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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 1 March 2004 with an application for Letters Patent number 531499 made by Auckland UniServices Limited.

Dated 16 March 2005.

Neville Harris
Commissioner of Patents, Trade Marks and Designs

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PROVISIONAL SPECIFICATION

NOVEL 1,2,4-BENZOTRIAZINE-1,4-DIOXIDES

We, **Auckland UniServices Limited,** a New Zealand company, of Level 10, 70 Symonds Street, Auckland, New Zealand do hereby declare this invention to be described in the following statement:

PT043956572

NOVEL 1,2,4-BENZOTRIAZINE-1,4-DIOXIDES

REFERENCE TO GOVERNMENT CONTRACT

The invention described herein was made in the course of work under grant or contract from the United States Department of Health and Human Services. The United States Government has certain rights to this invention.

TECHNICAL FIELD

The present invention provides a simplified set of characteristics that can be used to select 1,2,4 benzotriazine 1,4 dioxide compounds (TPZ analogues) with therapeutic activity against hypoxic cells in human tumour xenografts, and to further provide a novel class of 1,2,4-benzotriazine-1,4-dioxides (TPZ analogues) with predicted in vivo activity against tumours, to their preparation, and to their use as hypoxia-selective drugs and radiosensitizers for cancer therapy, both alone or in combination with radiation and/or other anticancer drugs.

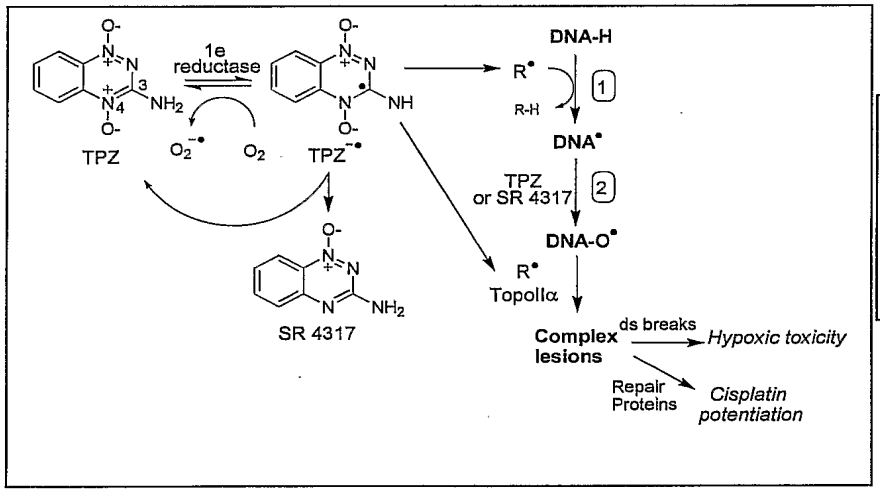
BACKGROUND TO THE INVENTION

It has been established that many human tumors contain a significant hypoxic fraction of cells [Kennedy et al., *Int. J. Radiat. Oncol. Biol. Phys.*, **1997**, *37*, 897; Movsas et al., *Urology*, **1999**, *53*, 11]. The presence of hypoxic cells arises because the extravascular transport (EVT) of oxygen is compromised due to an inefficient microvascular system within the tumor, which leads to large intercapillary distances and variable blood flow. Reduction of oxygen tension in tumors leads to radio-resistance. This reduction of oxygen tension causes up to a three-fold increase in radiation dose being required to kill anoxic tumour cells. A link has been identified between the presence of tumour hypoxia and failure of local control by radiation therapy [Brizel et al., *Radiother. & Oncol.*, **1999**, *53*, 113].

This phenomenon of tumour hypoxia has been exploited in the development of 'bioreductive drugs' [Brown et al., *Semin. Radiat. Oncol.* **1966**, *6*, 22; Denny et al., *Br. J. Cancer*, **1996**, *74 (Suppl. XXVII)* 32; Stratford & Workman, *Anti-Cancer Drug Des.*, **1998**, *13*, 519]. These agents are prodrugs that are selectively activated by enzymatic reduction in hypoxic cells, resulting in formation of a cytotoxin.

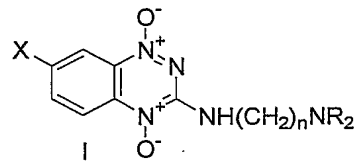
The 3-amino-1,2,4-benzotriazine 1,4-dioxides have been developed as bioreductive drugs for cancer therapy [Brown, *Br. J. Cancer*, **1993**, *67*, 1163-1170; Minchinton et al., *Int. J. Radiat. Oncol. Biol. Phys.* **1992,** *22*, 701-705 Kelson et al., *Anti-Cancer*

Drug Des., **1998**, *13*, 575; Lee et al., *WO 91/04028*, April 1991]. The lead compound of this class, tirapazamine (TPZ; SR 4233), is undergoing clinical trials in combination with radiotherapy and various chemotherapeutics, notably cisplatin [Denny & Wilson, *Exp. Opin. Invest. Drugs*, **2000**, *9*, 2889]. TPZ is activated by one electron reductases [Patterson et al., *Anti-Cancer Drug Des.* **1998** *13*, 541; Denny & Wilson, *Exp. Opin. Invest. Drugs*, **2000**, *9*, 2889] to form a radical that may be oxidized back to TPZ by molecular oxygen under aerobic conditions. Under hypoxic conditions the radical spontaneously generates an oxidizing radical(s) R$^{\bullet}$ (considered to be the hydroxyl radical [Daniels and Gates, *J. Am. Chem. Soc.*, **1996**, *118*, 3380-3385], and/or a benzotriazinyl radical [Anderson et al., *J. Am. Chem. Chem.* **2003**, *125*, 748-756]) which interact with DNA (and/or topoisomerase II)[Peters and Brown, *Cancer Res.*, **2002,** *62*, 5248-5253] to cause double-strand breaks and these correlate with cytotoxicity [Dorie et al., *Neoplasia,* **1999**, *1*, 461]. These features are illustrated in Scheme 1.



Scheme 1: Mechanism of action of TPZ

There have been only limited structure-activity studies on analogues of TPZ. Kelson et al. [*Anti-Cancer Drug Design*, **1998**, *13*, 575], Zeman et al. [*Int. J. Radiat. Oncol. Biol. Phys.*, **1989**, *16*, 977-981] and Minchinton et al. [*Int. J. Radiat. Oncol. Biol. Phys.*, **1992**, *22*, 701-705] disclosed compounds of type (I),

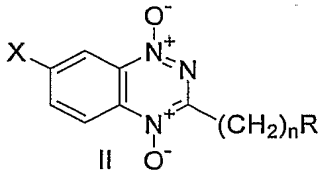


I

where X was H or an electron-withdrawing group, n was 2 or 3, and R was Me or Et. This paper showed that compounds with dialkylaminoalkyl side chains showed variable hypoxic selectivity in vitro. Compounds where X = H and having dialkylamino side

chains had a similar hypoxic cytotoxicty ratio to TPZ and comparable or inferior activity to TPZ in vivo.

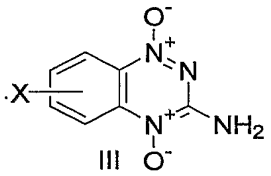
Hay and Denny [*Tet. Lett.*, **2002**, *43*, 9569] , Minchinton et al. [*Int. J. Radiat. Oncol. Biol. Phys.*, **1992**, *22*, 701-705] and Kelson et al. [*Anti-Cancer Drug Design*, **1998**, *13*, 575] described compounds of type II,



II

where X is H or hydroxyalkyl and R is OH or OMe. Kelson et al. [*Anti-Cancer Drug Design*, **1998**, *13*, 575] and Minchinton et al., [*Int. J. Radiat. Oncol. Biol. Phys.*, **1992**, *22*, 701-705] suggested that 3-alkyl compounds (X= H, n = 1,2 or 3, R = H and X= H, N = 2, R=OMe) were comparable to TPZ in vivo.

Finally, Hay et al. [Hay et al., *J. Med. Chem.* **2003**, *46*, 169] showed, for compounds of type III,



III

that there is an optimum range of one-electron reduction potential [E(1)] (between ca. –450 to –510 mV) for in vitro hypoxic selectivity. However, there was no clear relationship between the electron-withdrawing capability of the 7-substituent on the benzo ring and the reported biological activity.

Throughout this specification several abbreviations are used that require explanation and the following glossary is provided.

> IC_{50}: The concentration of drug (in micromolar, μM) to reduce cell numbers to 50% of those of control cell cultures grown under the same conditions but not exposed to drug.
>
> HCR: Hypoxic cytotoxicity ratio (the ratio of drug concentrations under aerobic and hypoxic conditions to produce equal cell survival (50%) determined by proliferation assay)

Kmet: First order rate constant for metabolism of a drug estimated at the C_{10} (see below)

C_{10}: the concentration required to produce one log of cell kill after exposure of cells to drug for one hour in clonogenic assays described in the methods (below).

PK: Pharmacokinetics. (Description of the variation in drug concentration with time (i.e. exposure) in a specified compartment or position within a tissue)

PD: Pharmacodynamics. (Description of the biological response to a drug)

PK/PD Model: Mathematical description of the relationship between drug exposure (PK) and biological response (PD).

Drawbacks of TPZ

Despite its advancement in clinical trials, several limitations of TPZ have been identified, including its relatively low solubility and poor therapeutic ratio [Rischin et al., Proc.Am.Soc.Clin.Oncol. **2003**, *22*, 495-496]. It is clear that the therapeutic ratio of TPZ in both preclinical (murine and human tumours) and clinical studies is low, and that there is a need for more tumour selective analogues. Preclinical studies have identified extravascular transport (EVT) as a factor that limits activity against hypoxic cells in tumours [Durand & Olive *Radiat. Oncol. Investig.* **1997**, *5*, 213; Durand & Olive, *Int. J. Radiat. Oncol. Biol. Phys.* **1992**, *22*, 689; Hicks et al, *Int. J. Radiat. Oncol. Biol. Phys.* **1998**, *42*, 641; Hicks et al, *Cancer Res.* **2003**, *63*, 5970; Kyle & Minchinton, *Cancer Chemother. Pharmacol.* **1999**, *43*, 213].

The EVT problem is thought to be particularly severe for bioreductive drugs, such as TPZ, for two reasons:

1. The target hypoxic cells are generally those most distant from the blood vessels
2. The metabolism of the bioreductive drug in the hypoxic tumour tissue will cause a continuously falling gradient of drug concentration through both the oxic and hypoxic tumour tissue which may not be overcome even with long infusion times.

However the same bioreductive metabolism which limits drug transport is also responsible for the cytotoxic effect of the drug [Baker et al. *Cancer Res.*, **1988**, *48*,

5947-5952; Siim et al, *Br. J. Cancer* **1996**, *73*, 952]. These competing effects of drug metabolism on EVT and cytotoxicity have been investigated using the multicellular layer model [Hicks et al, *Int. J. Radiat. Oncol. Biol. Phys.* **1998**, *42*, 641], as illustrated in Figure 1. Parameters determined by this model, together with single cell experiments to determine cytotoxicity and rates of metabolism [Hicks et al, *Cancer Res.* **2003**, *63*, 5970] and the oxygen dependence of cytotoxicity are used in a pharmacokinetic/ pharmacodynamic (PK/PD) model of cell killing in tumour tissue (as illustrated in Figure 2). The model and results obtained have demonstrated the need to optimise (rather than maximise) the rate of bioreductive metabolism. Figure 2 illustrates that high rates of metabolism will limit drug penetration and thus reduce cell kill in the hypoxic region, as well as decrease the differential in killing of hypoxic cells compared to well oxygenated cells. This is consistent with experimental results where high rates of metabolism limited activity in anoxic V79 multicellular spheroids [Durand & Olive *Int. J. Radiat. Oncol. Biol. Phys.* **1992**, *22*, 689] and anoxic HT29 MCL [Hicks et al., *Cancer Res.* **2003**, *63*, 5970], and resulted in a reduced hypoxic cytotoxic differential in SiHa human cervical tumours grown in SCID mice [Durand & Olive, *Radiat. Oncol. Investig.* **1997**, *5*, 213].

The PK/PD model for TPZ can be described as a distributed parameter model because it considers explicitly the spatial variation in parameter values (in other words it describes PK, and PD, as a function of position in tumour tissue). The main aspects of this distributed parameter PK/PD model have been disclosed in several publications (Hicks et al., *Int. J. Radiat. Oncol. Biol. Phys.* **1998**, *42*, 641; Hicks et al., *Cancer Res.* **2003**, *63*, 5970; Hicks et al., *Proc Am. Assoc. Cancer Res*, **2003**, Abstract #4561; Wilson et al., *Proc Am. Assoc. Cancer Res*, **2003**, Abstract #4570).

The key PK/PD relationship, as determined by investigating TPZ metabolism to its reduction product SR 4317 and cell killing as a function of TPZ concentration and time in anoxic stirred suspensions of HT29 colon carcinoma cells (Hicks et al., *Cancer Res.,* 2003), is described by:

Eqn 1: $$-\frac{d\log SF}{dt} = \gamma C \frac{dM}{dt}$$

This relationship shows that the rate at which cells are killed (on a log scale; SF = surviving fraction) is proportional to the rate of bioreductive drug metabolism (*M*, the amount of drug metabolised per unit intracellular volume) and to the drug

concentration, *C*. The constant of proportionality, γ, is a cell-line dependent parameter determined by fitting the model to data in clonogenic survival curves where drug concentrations are measured simultaneously. Under conditions of constant TPZ concentration, this approximates a concentration^2 $\times$ time dependence of log cell kill on TPZ exposure.

In order to describe PK/PD as a function of position in tumours, the above PK/PD model is extended to a spatially resolved (distributed parameter) model by incorporating the EVT properties (diffusion coefficient and rate of metabolism) of TPZ. In addition, because oxygen concentration in tumours varies as a function of distance from blood vessels, it is necessary to describe the relationship between O_2 concentration and rate of TPZ metabolism.

Simulation of TPZ diffusion into a tumour - in one dimension is illustrated in (Figure 3A. An O_2 concentration gradient in the one dimension planar tissue can be calculated numerically by solving the reaction-diffusion equation:

Eqn 2: $$\frac{\partial C_{O_2}}{\partial t} = D_{O2}\frac{\partial^2 C_{O_2}}{\partial x^2} - \frac{\partial C_{O_2}}{\partial t}$$

where C_{O_2} is the oxygen concentration in μM at position *x* and time *t*, using the diffusion coefficient and rate of metabolism in R3230Ac tumors (Dewhirst et al., *Cancer Res.*, **1994**, *54*, 3333; Secomb et al., *Adv.Exp.Med.Biol.* **1998**, *454*, 629) assuming an arteriolar input oxygen concentration of $[O_2]$ = 50 μM (38 mm Hg).

TPZ concentrations in the one dimensional tissue are calculated numerically from the reaction diffusion equation:

Eqn 3: $$\frac{\partial C}{\partial t} = D_{MCL}\frac{\partial^2 C}{\partial x^2} - \phi f([O_2])\frac{\partial M}{\partial t}$$

where *C* is the concentration of drug at position *x* and time *t*, D_{MCL} is the diffusion coefficient of drug in the multicellular layers, and ϕ is the cell volume fraction of the multicellular layer. Oxygen inhibits TPZ metabolism and this effect can be calculated according to the equation:

Eqn 4: $f([O_2]) = \left(\frac{K_{O_2}}{K_{O_2} + [O_2]} \right)$

where K_{O_2} is the O_2 concentration required for half maximal inhibition of TPZ metabolism.

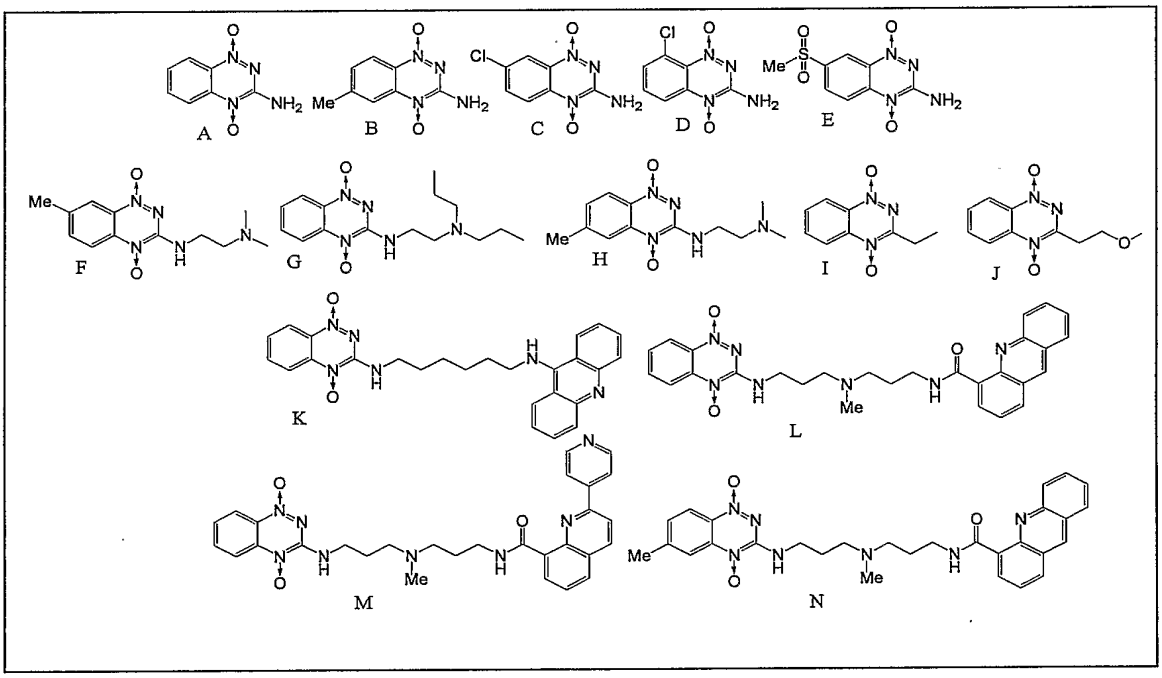
The above relationships (Eqn 1-4) define a PK/PD model for TPZ. The output of the model depends on the plasma PK of free drug (drug not bound to plasma proteins), which provides the input to the extravascular compartment, and on the geometry of the transport problem. Using this model the surviving fraction at each point in the tissue is calculated and the average log cell kill in the target hypoxic region evaluated. This is illustrated in Figure 2 for TPZ at its maximum tolerated in mice, together with simulations for a faster diffusing drug and an analogue with higher rates of metabolic activation.

This PK/PD model can be further extended to take into account the effects of diffusion in a three dimensional-3D microvascular environment, such as irregular vascular geometry, blood flow heterogeneity, and loss of oxygen and drug during transit through a capillary network. Such a 3D network is illustrated in Figure 3B and further described in (Hicks et al., *Proc Am. Assoc. Cancer Res*, **2003**, Abstract #4561; Wilson et al., *Proc Am. Assoc. Cancer Res*, **2003**, Abstract #4570). In this model similar equations as Eqns 1-4 are solved numerically using a Green's function method similar to that used for oxygen alone (Secomb et al., *Adv.Exp.Med.Biol.* **1998**, 454, 629).

In order to test the validity of this PK/PD model as a tool for predicting the antitumour activity of TPZ analogues, the inventors determined the key PK/PD parameters for 13 TPZ analogues, and TPZ itself, for the HT29 human colon carcinoma cell line. These parameters were then used to calculate the expected killing in hypoxic regions of HT29 tumours following a single intraperitoneal dose of the compounds at their maximum tolerated dose. The results of this calculation were then compared with measured killing of hypoxic cells in HT29 tumours, determined by administering the compounds immediately (within 5 min) after gamma irradiation (cobalt 60, 20 Gy) to sterilise oxygenated cells in the tumours. Tumours were removed 18 hours later and the number of surviving cells determined by clonogenic assay. The results for one compound **18** are shown in Example 36. The measured PK/PD parameters, model prediction (using the above 3D microvascular network), and experimentally

determined hypoxic cell kill is shown in Table 1, and the relationship between predicted and measured cell kill in Figure 4. The prediction is statistically highly significant ($p = 0.001$, Fisher exact test). Comparing the magnitude of predicted and observed response (Figure 4) also showed a highly significant linear correlation ($R^2 = 0.94$, $p < 0.001$ for a non-zero slope). If the extravascular transport component was excluded from the model, the R^2 for this regression was only 0.28 and the relationship was not statistically significant.

Table 1: Parameters of the PK/PD model for 14 benzotriazine di-N-oxides, model prediction of hypoxic cell killing in tumours, and measured hypoxic cell killing.



Cmpd	D_{MCL} cm^2s^{-1}	k_{met} min^{-1}	γ μM^2	AUC_f μM.hr	log kill (Pred)	log kill (Meas)	SE	Stat. Signif.
A	3.97×10^{-7}	0.60	2.21×10^{-5}	148.1	1.248	1.170	0.149	<0.01
B	5.43×10^{-7}	0.20	1.64×10^{-5}	188.7	1.923	2.165	0.149	<0.01
C	8.70×10^{-7}	3.92	3.71×10^{-5}	1.9	0.005	-0.007	0.184	ns
D	6.69×10^{-7}	10.03	2.25×10^{-6}	0.8	0.000	-0.154	0.064	ns
E	2.13×10^{-7}	1.00	1.40×10^{-6}	15.6	0.000	-0.028	0.083	ns
F	5.09×10^{-7}	2.54	6.84×10^{-5}	3.5	0.011	0.196	0.062	ns
G	1.71×10^{-6}	4.89	3.90×10^{-5}	7.2	0.048	0.248	0.072	ns
H	3.99×10^{-7}	1.52	1.10×10^{-4}	69.0	0.985	1.001	0.142	<0.01
I	2.61×10^{-6}	1.91	6.52×10^{-6}	140.6	1.116	0.886	0.132	<0.01
J	2.09×10^{-6}	2.01	2.90×10^{-4}	79.0	0.890	0.800	0.149	<0.05
K	6.17×10^{-7}	18.33	9.86×10^{-3}	0.042	0.000	0.009	0.102	ns
L	3.82×10^{-7}	9.18	1.30×10^{-3}	3.6	0.078	-0.051	0.039	ns
M	9.80×10^{-8}	9.13	3.04×10^{-3}	0.9	0.056	0.138	0.100	ns
N	1.05×10^{-7}	4.74	2.39×10^{-2}	1.3	0.092	0.027	0.121	ns

AUC_f = AUC of free drug

Log kill (Pred) = - $\log_{10}$ (hypoxic cell surviving fraction)

Log kill (Meas) = - $\log_{10}$ (hypoxic cell surviving fraction)

SE = standard error of log kill (Meas).

Stat. Signif. = statistical significance of log kill (Meas).

It is established by these studies that extravascular transport is a determinant of in vivo cytotoxicity and selectivity of benzotriazine di-N-oxides. These results confirm that the measurement of parameters such as IC50 and HCR in cell culture, alone, cannot be used as a basis to select compounds that are predicted to exhibit corresponding in vivo activity.

However, despite the elegance of these models and the highly statistically significant results achieved, one of the inherent difficulties with this approach is that it requires complex computational methods, and a detailed knowledge of a microvascular network geometry and blood flow. This information is not generally available. A further complication is that the prediction of in vivo activity also requires extensive in vitro investigation as well as extensive in vivo studies (determination of pharmacokinetics at a tolerable dose of the compound), which undermines the value of the predictive algorithm as a tool to select compounds that are predicted to exhibit corresponding in vivo activity.

It is therefore an object of the present invention to overcome some of these complexities by providing a simplified set of characteristics that can be used to select 1,2,4 benzotriazine 1,4 dioxide compounds (TPZ analogues) with therapeutic activity against hypoxic cells in human tumour xenografts, and to provide a method by which these characteristics can be assessed without administering TPZ analogues to animals and to further provide a novel class of TPZ analogues with predicted improved in vivo activity against tumours, relative to TPZ, or to at least provide the public with a useful choice.

DISCLOSURE OF THE INVENTION

In a first aspect the present invention provides a method of selecting one or more 1,2,4-benzotriazine-1,4-dioxides (one or more TPZ analogues) capable of in vivo hypoxia selective cytotoxicity, wherein said 1,2,4-benzotriazine-1,4-dioxide has each of the following characteristics

(a) a solubility greater than or about 2mM in αMEM; and

(b) an HT29 anoxic IC50 for a 4hr exposure to the 1,2,4-benzotriazine-1,4-dioxide of less than or about 40 μM; and

(c) an HT29 HCR greater than about 20; and

(d) a penetration half distance (PHD) greater than or about 27 μm, and

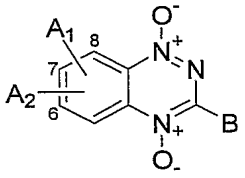
(e) the area under the plasma concentration time curve for free 1,2,4-benzotriazine-1,4-dioxide (unbound to plasma proteins), AUC_f, in μM.hr units is greater than about 3 times the HT29 anoxic IC_{50} measured in μM; (AUC_f/IC50 is greater than about 3 hr)

and wherein for said 1,2,4-benzotriazine-1,4-dioxide at least one of the characteristics (a) to (e) is superior to the equivalent characteristics of TPZ.

It is more preferred that the method of selection of the one or more TPZ analogues can be used to predict the in vivo activity of the one or more TPZ analogues without conducting animal studies.

In a further aspect, the invention provides a TPZ analogue selected by the method defined above.

Preferably, the TPZ analogue selected by the method defined above is a compound of Formula I or a pharmacologically acceptable salt thereof,



I

wherein

A_1 or A_2 represent a substituent at positions 6, 7 or 8 and are each independently selected from the following groups; H, R, or OR

wherein R represents a C_{1-4} alkyl optionally substituted with substituents selected from OMe, CO_2H, CO_2R^1 or NR^1R^1 and wherein each R^1 is independently selected from H or a C_{1-3} alkyl or the R^1R^1 substituents together form a cyclic alkyl amine selected from pyrrolidine, piperidine, 2,6-dimethylpiperidine, morpholine or azepane;

B represents NHR^2 or R^3;

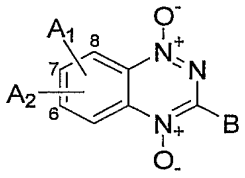
wherein R^2 is a C_{1-3} alkyl optionally substituted with substituents selected from OH, OMe, CN, CO_2H, CO_2R^4, or NR^4R^4

wherein R^3 is selected from a C_{1-3} alkyl optionally substituted with OH, OMe, or NR^4R^4,

wherein each R^4 is independently selected from H, a C_{1-3} alkyl or R^4R^4 together form a cyclic amine selected from pyrrolidine, piperidine, 2,6-dimethylpiperidine or morpholine; and

with the proviso that A_1 and A_2 both represent H only when B represents $NHCH_2CH_2CN$ or $NH_2CH_2CO_2CH_2CH_3$; and
with the further proviso that when A_1 represents H and A_2 represents 7-Me then B cannot represent $NH(CH_2)_2NMe_2$.

In a second aspect, the present invention provides a compound of Formula I or a pharmacologically acceptable salt thereof,



I

wherein
A_1 or A_2 represent a substituent at positions 6, 7 or 8 and are each independently selected from the following groups; H, R, or OR

wherein R represents a C_{1-4} alkyl optionally substituted with substituents selected from OMe, CO_2H, CO_2R^1 or NR^1R^1 and wherein each R^1 is independently selected from H or a C_{1-3} alkyl or the R^1R^1 substituents together form a cyclic alkyl amine selected from pyrrolidine, piperidine, 2,6-dimethylpiperidine, morpholine or azepane;

B is represents NHR^2 or R^3;

wherein R^2 is a C_{1-3} alkyl optionally substituted with substituents selected from OH, OMe, CN, CO_2H, CO_2R^4, or NR^4R^4

wherein R^3 is selected from a C_{1-3} alkyl optionally substituted with OH, OMe, or NR^4R^4,

wherein each R^4 is independently selected from H, a C_{1-3} alkyl or R^4R^4 together form a cyclic amine selected from pyrrolidine, piperidine, 2,6-dimethylpiperidine or morpholine; and

with the proviso that A_1 and A_2 both represent H only when B represents $NHCH_2CH_2CN$ or $NH_2CH_2CO_2CH_2CH_3$; and
with the further proviso that when A_1 represents H and A_2 represents 7-Me then B cannot represent $NH(CH_2)_2NMe_2$.

Preferably, in a compound of Formula I defined above A_1 represents Me, Et, OMe, or OEt; A_2 represents Me or OMe and B represents Me, Et, CH_2CH_2OH, CH_2CH_2OMe, $NHCH_2CH_2NMe_2$, $NHCH_2CH_2CH_2NMe_2$, $NHCH_2CH_2NEt_2$, $NHCH_2CH_2N$piperidine, $NHCH_2CH_2N$morpholine, $NHCH_2CH_2CH_2N$morpholine.

In a further embodiment preferably a compound of Formula I defined above A_1 represents $CH_2CH_2NMe_2$, $CH_2CH_2NEt_2$, CH_2CH_2Npiperidine, CH_2CH_2Nmorpholine, $CH_2CH_2CH_2N$morpholine, $OCH_2CH_2NMe_2$, $OCH_2CH_2NEt_2$, OCH_2CH_2Npiperidine, OCH_2CH_2Nmorpholine, $OCH_2CH_2CH_2N$morpholine and B represents Me, Et, NHMe, CH_2CH_2OH, CH_2CH_2OMe, NHEt, $NHCH_2CH_2OH$ or $NHCH_2CH_2OMe$.

In a third aspect the invention provides for the use in a method of therapy for treating cancer including the step of administering a compound of Formula I as defined above in a therapeutically effective amount to tumour cells in a subject.

Preferably the tumour cells are in a hypoxic environment.

It is preferred that the method of therapy further includes the step of administering radiotherapy to the tumour cells before, during or after the administration of the compound of Formula I as defined above to the tumour cells.

It is preferred that the method of therapy further includes the step of administering one or more chemotherapeutic agents to the tumour cells before, during or after the administration of the compound of Formula I as defined above to the tumour cells.

In a fourth aspect the invention provides for the use in the manufacture of a medicament of a therapeutically effective amount compound of a Formula I as defined above for the treatment of tumour cells in a subject.

Preferably the tumour cells are in a hypoxic environment.

While these compounds will typically be used in cancer therapy of human subjects, they can be used to target tumour cells in other warm blooded animal subjects such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

A "therapeutically effective amount", is to be understood as an amount of a compound of Formula I as defined above or a compound of Formula I' as defined above or a mixture thereof that is sufficient to show benefit to a patient. The actual amount, rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment is within the responsibility of general practitioners and other medical doctors.

A hypoxic environment is to be understood as either an *in vitro* or *in vivo* environment having a poorer blood supply and lower oxygen tension than normal tissues.

It is to be understood that the compound of Formula I can be administered alone or in combination with other chemotherapeutic agents or treatments, especially radiotherapy, either simultaneously or sequentially dependent upon the condition to be treated.

Preferred chemotherapeutic agents can be selected from:
Cisplatin or other platinum-based derivatives,
Temozolomide or other DNA methylating agents,
Cyclophosphamide or other DNA alkylating agents,
Doxorubicin, mitoxantrone, camptothecin or other topoisomerase inhibitors,
Methotrexate, gemcitabine or other antimetabolites.

In a fifth aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of formula I or a mixture thereof, a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

The pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which can be oral, or by injection, such as cutaneous, subcutaneous, or intravenous injection.

Pharmaceutical compositions for oral administration can be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvent. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol,

propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as gelatin.

For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has a suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers antioxidants and/or other additives may be included as required.

It is to be recognised that certain compounds of the present invention may exist in one or more different enantiomeric or diastereomeric forms. It is to be understood that the enantiomeric or diasteriomeric forms are included in the above aspects of the invention.

The term pharmacologically acceptable salt used throughout the specification is to be taken as meaning any acid or base derived salts formed from hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicyclic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isoethonic acids and the like and potassium carbonate sodium or potassium hydroxide ammonia, triethylamine, triethanolamine and the like.

Further aspects of the present invention will become apparent with reference to the following detailed description, the Synthetic Schemes, the Examples; and the Figures, which are given by way of example only, where

Figure 1 shows schematically a method for quantifying extravascular transport (EVT) properties of compounds of Formula I using multicellular layers (MCL). **A**: Diagram of a Millicell CM culture insert used for growing MCLs on a porous, collagen-coated Teflon membrane. **B**: H&E-stained transverse section of an HT-29 MCL three days after initiating growth with 10^6 cells. The Teflon membrane is ca 30 μm thick. **C**: Diffusion apparatus for measurement of transport through MCLs. **D**: Diffusion of TPZ (100 μM) through well oxygenated (open circles) and a 2-fold reduction in this diffusion in poorly oxygenated (filled circles) HT29 MCLs of equivalent thickness (141 ± 5 μm). Concentrations are normalized to the initial concentration in the donor compartment (C_o). The Lines are fits to a reaction-diffusion model. Also shown is the

diffusion of compound #20 through a well oxygenated MCL of a similar thickness illustrating the effect of raising the diffusion coefficient from 4×10^{-7} cm^2s^{-1} to 1.4×10^{-6} cm^2 s^{-1}.

Figure 2. Illustrates the sensitivity of hypoxic cell killing in tumours to changes in diffusion coefficient of TPZ analogues, simulated using a spatially resolved 1D PK/PD model. The solid lines are based on the measured rate of metabolism of TPZ by HT29 cells, and the dashed line on a two-fold higher rate of metabolism. The *Left Panel* of Figure 2 shows steady-state concentration gradients as fractions of the plasma concentration, (C_p = 50 μM) for O_2, TPZ and a TPZ analogue with 6-fold higher diffusion coefficient assuming the same rate of metabolism as for TPZ. The *Right Panel* of Figure 2, shows predicted cell killing for the same two compounds. The cytotoxicity parameter (γ) is assumed to have the same value as that found experimentally for TPZ. The numbers on the graph refer to the following situations:

1. A 2-fold increase in the rate of metabolism (and hence 2-fold increase *in vitro* cytotoxic potency) results in no significant increase in killing in the hypoxic region (O2 < 4 μM) but gives a 2-fold increase in killing of oxygenated cells near blood vessels. This change is therefore predicted to be therapeutically unfavourable, and demonstrates the importance of optimising (rather than maximising) rates of metabolic reduction of TPZ analogues.
2. A 6-fold increase in the diffusion coefficient, with no increase in the rate of metabolism and hence no increase in *in vitro* cytotoxic potency, results in a 2-fold increase in cell killing in the hypoxic zone without any undesirable increase in cell killing in the oxic zone. This 2-fold increase in hypoxic selectivity *in vivo* is predicted to be therapeutically favourable.
3. A 6-fold increase in the diffusion coefficient together with a 2-fold increase in the rate of metabolism (and hence a 2-fold increase in *in vitro* potency) results in a 3-fold increase in cell killing in the hypoxic zone and a 2-fold increase in *in vivo* hypoxic selectivity.

Figure 3 illustrates the geometry used in the PK/PD model of extravascular transport in tumours. **A**: illustrates the 1D diffusion into a planar tissue region between two capillaries with a line showing the falling oxygen concentration gradient from the capillary to the centre of the region. **B**: illustrates the 3D diffusion in a mapped microvascular network in a 230 × 500 × 500 μm region in a R3230Ac tumour.

Figure 4: Shows schematically a predicted (PK/PD model) versus measured kill of hypoxic cells in HT29 tumors (i.e. additional kill for drug immediately after radiation, relative to radiation only).

Detailed Description of the Invention

Impeded extravascular transport has been previously identified as limiting the in vivo cytotoxicity and selectivity of many 1,2,4-benzotriazine-1,4-dioxides including TPZ. It is also recognised that some of the limitations of TPZ are because it is metabolised too quickly before it reaches its desired hypoxic destination. There is a complex relationship between diffusion, metabolism and in vivo activity and in order to select an improved TPZ analogue there is a need to optimize (rather than maximize) the rate of metabolism of a TPZ analogue in vivo simultaneously with the other transport and potency properties.

The inventors have now discovered a simplified but specific set of characteristics that can be used to select a TPZ analogues with therapeutic activity against hypoxic cells in human tumour xenografts, and a method by which these characteristics can be assessed without administering compounds to animals.

The determination of the desired characteristics came about by closely studying and measuring the parameters influencing and determining the extravascular transport and potency of hypoxia selective cytotoxins in vitro, PK/PD modelling, and comparison with in vivo cell killing in the HT29 excision assay the modelling and computational methods used in selecting a TPZ analogue having predicted optimised metabolism in vivo. The desired characteristics are interrelated and the limits have been carefully selected to ensure that compounds having undesirable characteristics, for example where the TPZ analogue is not sufficiently selective in its cytotoxicity under hypoxic conditions, are excluded.

The selection of the specific characteristics for a suitable TPZ analogue are as follows:

1 a solubility greater than or about 2mM in αMEM; and

2 an HT29 anoxic IC50 for a 4hr exposure to the 1,2,4-benzotriazine-1,4-dioxide of less than or about 40 μM; and

3 an HT29 HCR greater than about 20; and

4 a penetration half distance (PHD) greater than or about 27 μm, and

5 the area under the plasma concentration time curve for free 1,2,4-benzotriazine-1,4-dioxide (unbound to plasma proteins), AUC_f, in μM.hr units is greater than about 3 times the HT29 anoxic IC_{50} measured in μM; (AUC_f/IC50 is greater than about 3 hr)

It is to be appreciated that while the characteristics have been selected to predict TPZ analogues that are active against HT29 tumours in mice, it is expected that such TPZ analogues will also be active against hypoxic tumour cells in humans or at least will have an increased probability of having such activity relative to other TPZ analogues that do not meet all of the characteristics (a) to (e) above.

Although the threshold for each parameter is set at a value less favourable than the specific value determined for TPZ, these rules still make it possible to successfully predict those compounds with significant activity against hypoxic cells in human tumour xenografts in mice. For example, if a compound satisfies all of the characteristics (a) to (e) it is very likely that this compound will provide optimised in vivo metabolism relative to TPZ.

To exemplify the invention, Table 2 provides demonstration that the above characteristics correctly identify the in vivo active compounds in the validation set of 14 compounds that have been assayed in vivo (described in Table 1 above).

Table 2: Ability of the characteristics to be used to select in vivo active compounds in the validation set of TPZ analogues. For compound structures, refer Table 1. Failure to meet each characteristic limit is identified in bold. Compounds satisfying all the desired characteristic conditions are shown with a grey background. All 5 of the 14 compounds meeting the desired characteristics show significant activity against hypoxic cells in HT29 tumours.

Compound	Solubility	IC50	HCR	PHD	AUC /IC50	HT29 EA	EA	Characteristics (a) to (e)
	>2 mM	**<40µM**	**>20**	**>27µm**	**>3**	**> 0.6**		**achieved**
A	8.90	5.81	71.0	43.7	25.5	1.170	Active	5/5
B	3.02	12.90	35.8	89.5	14.6	2.165	Active	5/5
C	**0.09**	0.73	28.5	**25.3**	**2.6**	-0.007	Inactive	2/5
D	2.22	0.55	**13.6**	**13.9**	**1.4**	-0.154	Inactive	2/5
E	**0.49**	13.62	**13.0**	**24.8**	**1.1**	-0.028	Inactive	1/5
F	38.70	1.07	34.3	**24.0**	3.2	0.196	Inactive	4/5
G	26.00	3.09	71.0	31.8	**2.3**	0.248	Inactive	1/5
H (18)	52.00	4.06	215.0	27.5	17.0	1.001	Active	5/5
I	14.50	3.80	37.4	62.8	37.0	0.886	Active	5/5
J	22.00	3.49	49.4	54.8	22.6	0.800	Active	5/5
K	**0.025**	0.02	38.4	**9.9**	**2.7**	0.009	Inactive	2/5
L	**0.45**	0.04	154.0	**11.0**	84.2	-0.051	Inactive	3/5
M	3.80	0.11	119.0	**5.6**	8.0	0.138	Inactive	4/5
N	32.40	0.22	134.0	**25.3**	5.9	0.027	Inactive	4/5

EA = HT29 excision assay (measured log cell kill)

Determination of Characteristics (a) to (e)

(a) Determination of Solubility

Solubility is determined in laboratory culture medium (alpha minimal essential medium (αMEM) with 5% foetal bovine serum) saturated with 5% carbon dioxide at pH 7.4, by addition of excess compound and sonication at ambient temperature for 15 minutes. Alternatively the compound is diluted from a concentrated stock solution in DMSO into culture medium to give a final DMSO concentration <1%. The mixture is centrifuged at 13,000 rpm for 6 minutes and the concentration of drug in the supernatant solution is then determined by HPLC using a standard reference solution in a suitable solvent.

(b) and (c) Determination of Cytotoxicity

Evaluation of the cytotoxicity of compounds by proliferation assay (IC_{50}) under aerobic and hypoxic conditions.

Compounds representative of the invention were evaluated under both aerobic and hypoxic conditions in a proliferation assay (IC_{50}) using the human colon carcinoma cell line HT-29 as previously described [Hay et al, *J. Med. Chem*, **2003**, *46*, 169]. For each experiment, compounds were simultaneously tested under both oxic and hypoxic conditions and included TPZ as an independent internal control at the front and back of the assay. In all cases, a 8-methyl-5-nitroquinoline derivative was used as a second internal control to confirm that strict hypoxia was present during the experiment [Siim et al., *Br. J. Cancer* **1994**, *70*, 596. After exposure to compounds for 4 hrs, cells were washed with fresh medium and grown for a further 5 days before staining with sulforhodamine B as described previously [Wilson et al., *J. Med. Chem.* **1989**, *32*, 31] and IC_{50} values determined.

IC_{50} = The concentration of drug (in micromolar) to reduce viable cell numbers to 50% of those of control cell cultures grown on the same plate but not exposed to drug.

HCR = Hypoxic cytotoxicity ratio is defined as the ratio of IC_{50} values under aerobic and hypoxic condition

(d) Determination of PHD

For a TPZ analogue to selectively kill hypoxic cells in vivo it must be capable of transport to the hypoxic region. Transport limitations are the result of the competition between diffusion (governed by the diffusion coefficient D_{MCL} cm^2s^{-1}) and bioreductive metabolism (measured by the first order rate constant k_{met} in s^{-1}). This competition may be summarised as the Penetration Half Distance (*PHD*), which is the distance into a plane one dimensional anoxic tissue region where the drug concentration falls to half of its external value, and is calculated by

$$PHD = \ln(2)\sqrt{\frac{D_{MCL}}{k_{met}}}$$

where D_{MCL} is the drug diffusion coefficient in HT29 MCL in units cm^2s^{-1} and k_{met} is the estimated first order rate constant for hypoxic drug metabolism in HT29 MCL at a drug concentration approximating the C_{10} value in units of s^{-1}.

PHD for compounds representative of the invention were evaluated as described. The requirement that

$$PHD \geq 27\ \mu m$$

ensures adequate extravascular transport by setting an upper bound on k_{met} as a function of D_{MCL} , i.e., k_{met} must be less than or equal to $\frac{2}{3} D_{MCL} \times 10^5\ s^{-1}$. The lower bound on k_{met} is implied by the IC50 and HCR conditions which ensure that the rate of metabolism under hypoxia is high enough to provide potent and selective hypoxic cell killing.

The parameters k_{met} and D_{MCL} can be estimated by measurement or by calculation as illustrated below.

PHD = is the distance into a plane one dimensional anoxic tissue region where the drug concentration falls to half of its external value
MCL = multicellular layer
D_{MCL} = the diffusion coefficient of the drug in HT29 multicellular layers (see below)
k_{met} = the rate constant for bioreductive metabolism at the cell density in HT29 multicellular layers

<u>Determination of the diffusion coefficient in HT29 multilayers</u>

The diffusion coefficients in HT29 MCL (D_{MCL}) of compounds representative of the invention were determined either by:

1. Measurement of drug diffusion in HT29 MCL (grown in culture inserts and seeded at 1×10^6 cells per insert and grown for 3-4 days) in a 2 chamber diffusion apparatus containing culture medium with measurement from both the donor and receiver compartments and gassing at ≥ 20% O2 to suppress bioreductive metabolism as described in Hicks et al (*Cancer Res.* **2003**, 63, 5970-5977). Samples of medium are taken at intervals, drug concentrations determined by HPLC or LCMS and the concentration-time profile was fitted to Fick's second law of diffusion and the differential equation was solved numerically to obtain the estimate of D_{MCL}.

   OR

2. Calculated from the logistic regression equation

$$\log(D_{MCL}) = y_0 + g + d \times \log M_r + \frac{a}{1+\exp\left(-\frac{\log P_{7.4} - x_0 + e \times HD + h \times HA}{b}\right)}$$

   where

   a. Log $P_{7.4}$ is the base 10 logarithm of the octanol-water partition coefficient of the compound at pH 7.4 measured or calculated using the techniques described below
   b. *HD* is the number of hydrogen bond donors (which is the sum of all NH- and OH-groups)
   c. *HA* is the number of hydrogen bond acceptors (which is the sum of all N- and O-atoms)
   d. M_r is the molecular weight of the non-ionised drug

and *a*, *g*, *d*, *a*, x_0, *e*, *h*, and *b* are regression coefficients as outlined in the table.

Parameters

	Estimate	SE	CV(%)	p
a	1.0955	0.0746	6.81	<0.0001
b	0.6452	0.0994	15.41	<0.0001
d	-0.4731	0.1027	21.71	<0.0001
e	-0.9602	0.0916	9.54	<0.0001
x_0	-3.5797	0.3541	9.89	<0.0001
y_0	-5.5183	0.2506	4.54	<0.0001
h	-0.3810	0.0490	12.86	<0.0001

Values of the coefficient *g* are cell line dependent; this value has been determined as 0.3051 (SE 0.0427, CV 13.99%, p <0.001) for SiHa MCLs, and is set at zero for HT29 MCLs.

Determination of the rate of metabolism

The apparent first order rate constants for anoxic metabolism in HT29 cells (k_{met}) of compounds representative of the invention were either:

1. Estimated at the C_{10} experimentally by incubating stirred single cell suspensions (typically 10 ml at 2×10^6/ml of HT29 cells derived by trypsinisation of multicellular spheroids) in αMEM without serum in 20 ml bottles under flowing 5% CO_2/N_2 for 90 min, then introducing the compound using deoxygenated DMSO stock solutions to give a range of final drug concentrations. Samples (0.5 ml) were removed at intervals (typically 5 min, 30 min, 1,2,3 hr), washed by centrifugation, and plated to determine the number of clonogenic survivors as described by Hicks et al Cancer Res. 2003 *63*, 5970. The concentration of compound giving 1 log of kill at 1 hr (C_{10}) was estimated by interpolation. Additional samples taken at the same times were centrifuged to remove cells, and supernatant stored at –80°C for subsequent HPLC or LCMS analysis. The concentration of compound in the extracellular medium was plotted against time and the concentration closest to the C_{10} was used to estimate the first order rate constant. This was scaled to MCL cell density as described in Hicks et al, *Cancer Res.* **2003**, *63*, 5970 to obtain k_{met}. Cell viability was determined with a hemocytometer at the end of drug exposure by staining with 0.4% trypan blue to ensure metabolic viability was >75%. TPZ (30 μM) was included in each experiment as a reference compound.O_2 in solution was measured using an OxyLite O_2 luminescent fiber optic probe (Oxford Optronix Ltd, UK) to ensure severe hypoxia (< 0.1 μM).

OR

2. Calculated by regression against the measured one electron reduction potential E(1) to using the following equation

$$\log k_{met} = 4.7220549 + 0.0106557 \times E(1) \; (R^2 = 0.796)$$

where b[0] and b[1] are the regression coefficients outlined in the following table

Determination of physicochemical parameters

Physicochemical parameters of compounds representative of the invention were determined as follows.

1. log $P_{7.4}$

The base 10 logarithm of the octanol-water partition coefficient was determined either

1. Experimentally by a modified shake flask method as described in Siim et al [Siim et al, *Biochem.Pharmacol.* **2000**, *60*, 969] by partitioning of drug between phosphate buffered saline and analytical grade 1-octanol at 22± 2 °C with measurement of both aqueous and octanol phases by HPLC or LC/MS after equilibrium is reached .

OR

2. By calculation using proprietary software ACD log *D* (Advanced Chemistry Development Inc, Toronto, Canada with inclusion of a training set of compounds for which have log $P_{7.4}$ has been measured as described above.

2. E(1)

The one-electron reduction potential (E(1)) was determined either

1. Experimentally, using pulse radiolysis [Wardman, *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637; Anderson et al, *Brit. J. Cancer* **1996**, *27*, S48] performed on a Dynaray 4 (4 MeV) linear accelerator (200 ns pulse length with a custom-built optical radical detection system. *E*(1) values were determined in anaerobic aqueous solutions containing 2-propanol (0.1 M) buffered at pH 7.0 (10 mM phosphate) by measuring the equilibrium constant [Meisel & Czapski, *J. Phys. Chem.* **1975**, *79*, 1503] for the electron transfer between the radical anions of the compounds and the appropriate viologen or quinone reference standard. Data were obtained at three concentration ratios.

OR

2. By calculation. For monosubstituted compounds, E(1) may be estimated by regression using the following equations (Hay et al., J.Med.Chem. 2003, 46, 169-182)

3-sub: $E(1)/mV = -348 + 161\,\sigma_p$ n=5 r^2=0.975 F= 161

5-sub: $E(1)/mV = -453 + 161\sigma_m$ n=7 r^2=0.976 F=160

6-sub: $E(1)/mV = -454 + 282\sigma_p$ n=10 r^2=0.987 F=596

7-sub: $E(1)/mV = -424 + 171\sigma_p$ n=10 r^2=0.933 F=111

8-sub: $E(1)/mV = -492 + 287\sigma_m$ n=10 r^2=0.946 F=106

(e) Determination of free drug AUC from Plasma pharmacokinetics (PK)

The area under the concentration time curve for free drug (AUC_f) in mouse plasma at the maximum tolerated dose (MTD) was determined either experimentally or by calculation.

1. Experimental Determination of Plasma pharmacokinetics (PK)

A. Determination of Maximum Tolerated Dose (MTD)

The compound was formulated in a suitable vehicle (e.g. 0.9% saline, 5% DMSO in 0.9% saline) and administered intraperitoneally (i.p.) as single dose to CD-1 nude mice in a dose-escalating format using 1.33-fold dose increments. The mice were weighed and observed at regular intervals and the MTD defined as the highest dose that does not cause lethality or severe morbidity or unacceptable toxicity (e.g. a weight loss of greater than 15% of the starting weight in any individual animal) in a group of 3-6 mice.

B. Plasma pharmacokinetics (PK)

The compound was administered to CD-1 nude mice in a suitable formulation as a single dose (i.p.) at the MTD. Blood samples were collected by retro-orbital sinus bleed or cardiac puncture after which the mouse was culled, or by serial bleeding from the tail vein. Typical time points were 15, 30, 60, and 120 min after administration. Blood was collected in a heparinised container and centrifuged to collect the plasma. The plasma concentration of the compound was determined by HPLC or LCMS using suitable sample preparation and analytical methods. Calibration was done with internal or external standards. The area under the concentration-time curve (AUC) for total (free plus bound) drug was calculated using the linear/log trapezoidal rule and extrapolation to infinity OR standard non-compartmental (PK) modelling.

C. Determination of plasma protein binding

Plasma protein binding was measured by the determination of the free fraction (FF) by equilibrium dialysis at 37°C in 50% (v/v) mouse plasma in phosphate-buffered saline (pH 7.4), using a single drug concentration at or near the observed or extrapolated maximum concentration in plasma (C_{max}). Compound concentrations after dialysis were determined by HPLC or LCMS. The plasma protein FF was then used to estimate binding in 100% mouse plasma using the relationship:

FF(100% plasma) = 0.5 FF(50% plasma)

D. Determination of the AUC for free drug

The AUC for the free drug (AUC_f) was estimated using the equation:

$$AUC_f = \text{FF(100\% plasma)} \times AUC$$

2. Calculation of AUC_f

AUC_f for the compound administered as a single dose (i.p.) to CD-1 nude mice at the MTD can be estimated using the following regression equation:

$$\text{Log}_{10}(AUC_f) = a + b \times \text{logP}_{neutral} + c \times \log_{10}(IC_{50}) + d \times \log_{10}(\text{HCR}) + e \times \log_{10}(D_{MCL}) + f \times \log_{10}(k_{met}) + g \times (\text{logP}_{neutral})^2$$

where the coefficients are described in the following table:

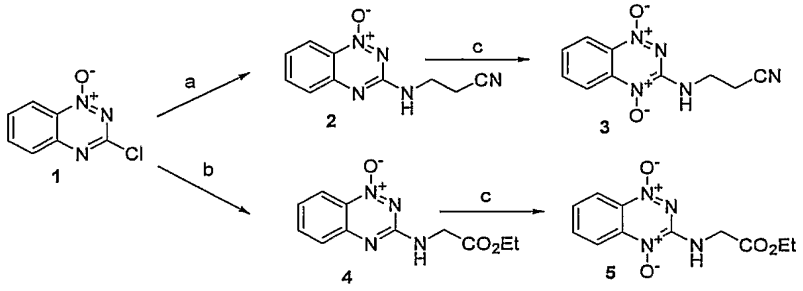
	Coefficient	Std. Error	CV%	P
a	0.3810	0.3573	93.7795	0.3091
b	-0.1411	0.1208	85.6130	0.2676
c	1.3007	0.1794	13.7926	<0.0001
d	0.9457	0.1771	18.7269	0.0002
e	0.8940	0.2069	23.1432	0.0012
f	-0.1739	0.1937	111.3859	0.3885
g	-0.3068	0.0728	23.7288	0.0015

N=18 R^2=0.9556 SE=0.1877 F=39.4382 P<0.0001

Methods for preparing compounds of Formula I of the invention.

Reaction of chloride **1** with aminopropionitrile or glycine etheyl ester gave 1-oxides **2**, **4** (Scheme 1) which were oxidised to the corresponding 1,4-dioxides **3** and **5** with MCPBA.

Scheme 1



Reagents:

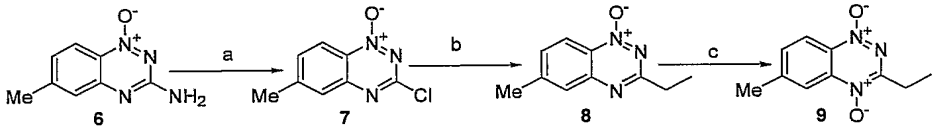
a) aminopropionitrile fumarate, Et_3N, DME;

b) glycine ethyl ester, Et_3N, DME;

c) MCPBA, DCM.

Diazotisation of amine **6** [Hay et. al., *J. Med. Chem.* **2003**, 46, 169] in trifluoroacetic acid and chlorination of the intermediate phenol gave chloride **7** (Scheme 2). Stille reaction of chloride **7** with tetraethyltin and $Pd(PP_3)_4$ gave 1-oxide **8**, which was oxidised with trifluoroperacetic acid to 1,4-dioxide **9**. Reaction of **6** with allyltributyltin under Stille reaction conditions gave 1-oxide **10** (Scheme 3). Ozonolysis of **10** with a reductive workup gave alcohol **11** which was oxidized to give 1,4-dioxide **12**. Alcohol **11** was methylated with TMS-diazomethane and HBF_4 to give ether **13**. Oxidation with trifluoroperacetic acid gave 1,4-dioxide **14**.

Scheme 2



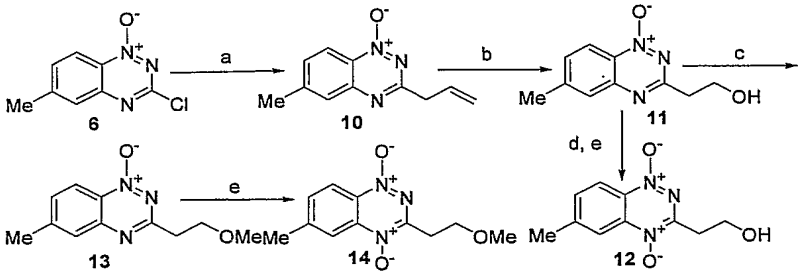
Reagents:

a) $NaNO_2$, TFA; then $POCl_3$, DMF;

b) Et_4Sn, $Pd(PPh_3)_4$, DME;

c) CF_3CO_3H, $CHCl_3$.

Scheme 3



Reagents:

a) $nBu_3Snallyl$, $Pd(PPh_3)_4$, DME;

b) O_3, DCM, MeOH, then $NaBH_4$, EtOH;

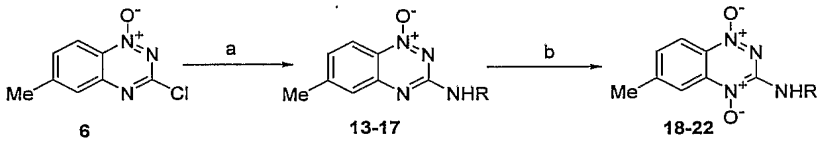
c) $TMSCH_2N_2$, HBF_4, DCM;

d) $(CF_3CO)_2O$, pyridine, DCM;

e) CF_3CO_3H, $CHCl_3$.

Nucleophilic displacement of **6** with a variety of amines gave 1-oxides **13-17** which underwent selective aromatic *N*-oxidation under acidic conditions to give 1,4-dioxides **18-22** (Scheme 4).

Scheme 4



Reagents:

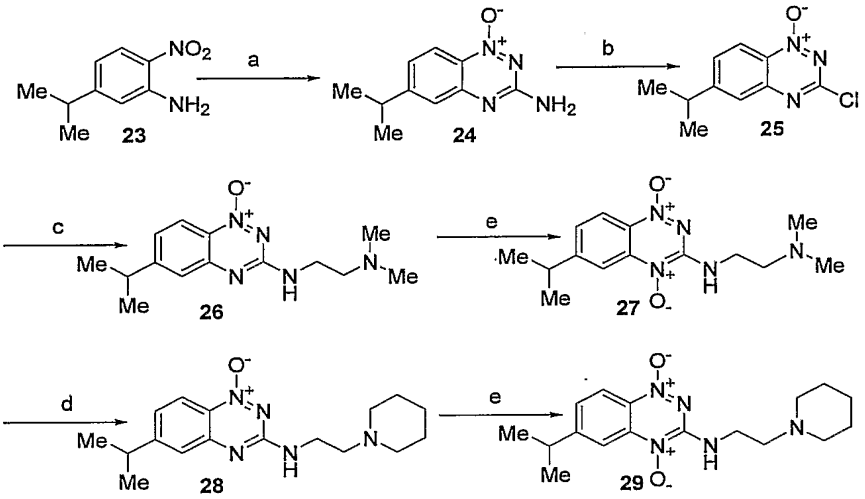
a) amine, DME;

b) CF_3CO_2H, CF_3CO_3H, DCM.

Reagent	1-oxide	1,4-dioxide	R =
$NH_2CH_2CH_2NMe_2$	**13**	**18**	$-CH_2CH_2NMe_2$
$NH_2CH_2CH_2NEt_2$	**14**	**19**	$-CH_2CH_2NEt_2$
$NH_2CH_2CH_2NPr_2$	**15**	**20**	$-CH_2CH_2NPr_2$
$NH_2CH_2CH_2N$piperidine	**16**	**21**	$-CH_2CH_2N$piperidine
$NH_2CH_2CH_2N$-2,6-Me_2piperidine	**17**	**22**	$-CH_2CH_2N$-2,6-Me_2piperidine

Condensation of 5-isopropyl-2-nitroaniline (**23**) [Prasad, J. V. N. V. *Org. Lett.* **2000**, *2*, 1069] and cyanamide gave the 1-oxide **24** which underwent diazotisation and chlorination to give chloride **25** (Scheme 5). Nucleophilic displacement of chloride **25** with amines gave the 1-oxides **26** and **28** which were oxidised to the corresponding 1,4-dioxides **27** and **29**.

Scheme 5



Reagents:

a) NH_2CN, HCl; then NaOH;

b) $NaNO_2$, TFA; then $POCl_3$, DMF;

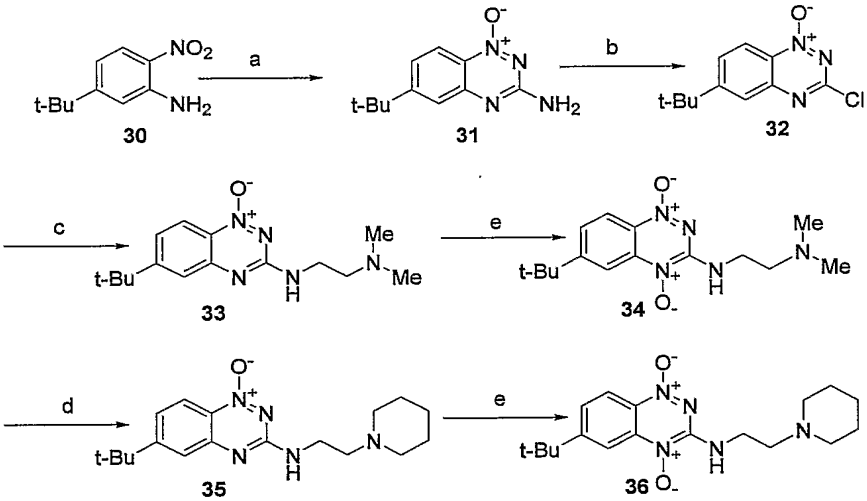
c) $NH_2CH_2CH_2NMe_2$, DME;

d) $NH_2CH_2CH_2N$piperidine, DME;

e) CF_3CO_2H, CF_3CO_3H, DCM.

Condensation of 4-*tert*-butyl-2-nitroaniline (**30**) [Seko, S, et al, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1437] and cyanamide gave the 1-oxide **31** which underwent diazotisation and chlorination to give chloride **32** (Scheme 5). Nucleophilic displacement of chloride **32** with amines gave the 1-oxides **33** and **35** which were oxidised to the corresponding 1,4-dioxides **34** and **36**.

Scheme 6



Reagents:

a) NH_2CN, HCl; then NaOH;

b) $NaNO_2$, TFA; then $POCl_3$, DMF;

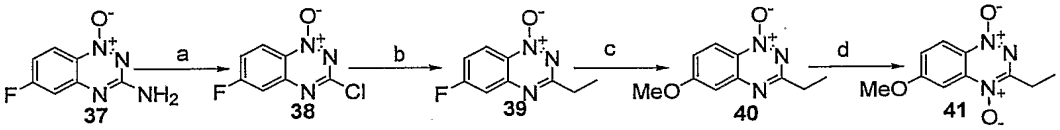
c) $NH_2CH_2CH_2NMe_2$, DME;

d) $NH_2CH_2CH_2N$piperidine, DME;

e) CF_3CO_2H, CF_3CO_3H, DCM.

Diazotisation of amine **37** (Hay et. al., *J. Med. Chem.* **2003**, *46*, 169) in trifluoroacetic acid and chlorination of the intermediate phenol gave chloride **38** (Scheme 7). Stille reaction of chloride **38** with tetraethyltin and $Pd(PP_3)_4$ gave 1-oxide **39** which readily underwent nucleophilic displacement with methoxide to give 1-oxide **40** and oxidation to the 1,4-dioxide **41**.

Scheme 7



Reagents:

a) $NaNO_2$, TFA; then $POCl_3$, DMF;

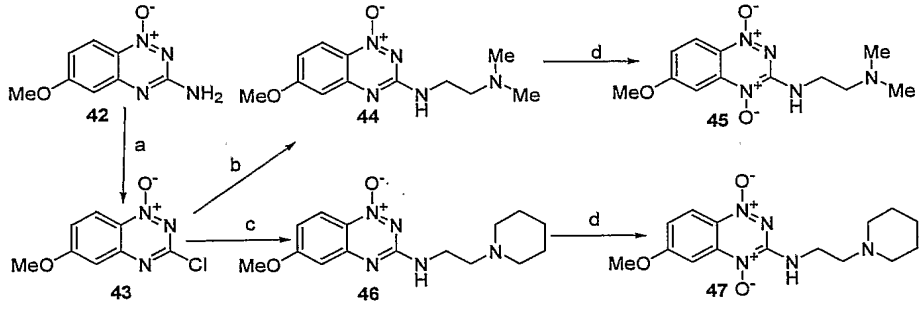
b) Et_4Sn, $Pd(PPh_3)_4$, DME;

c) NaOMe, MeOH;

d) CF_3CO_3H, $CHCl_3$.

Diazotisation of amine **42** (Hay et. al., *J. Med. Chem.* **2003**, 46, 169) in trifluoroacetic acid and chlorination of the intermediate phenol gave chloride **43** (Scheme 8). Nucleophilic displacement of chloride **43** with amines gave the 1-oxides **44** and **46** which were oxidised to the corresponding 1,4-dioxides **45** and **47**.

Scheme 8



Reagents:

a) $NaNO_2$, TFA; then $POCl_3$, DMF;

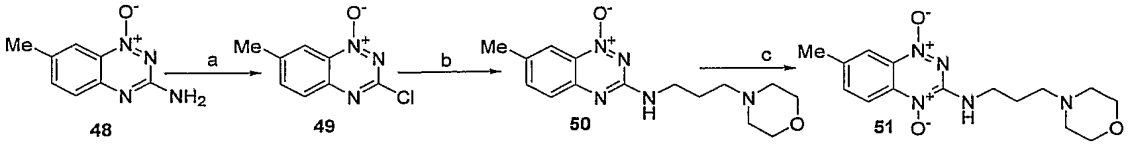
b) $NH_2CH_2CH_2NMe_2$, DME;

c) $NH_2CH_2CH_2N$piperidine, DME;

d) CF_3CO_2H, CF_3CO_3H, $CHCl_3$.

Diazotisation of amine **48** (Hay et. al., *J. Med. Chem.* **2003**, 46, 169) and chlorination of the intermediate phenol gave chloride **49** (Scheme 9). Nucleophilic displacement of chloride **49** with 3-(4-morpholinyl)propylamine gave the 1-oxide **50** which was oxidised to 1,4-dioxide **51**.

Scheme 9



Reagents:

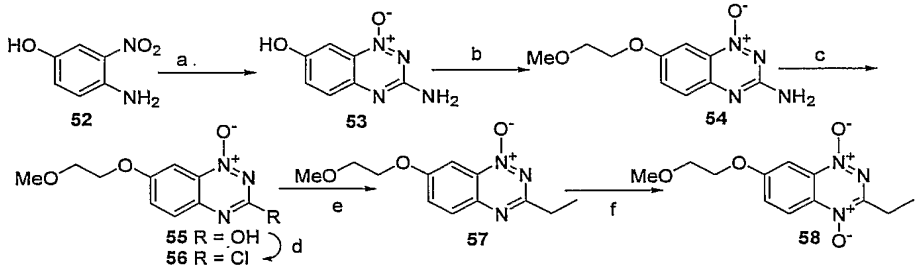
a) $NaNO_2$, aqueous HCl; then $POCl_3$, DME;

b) $NH_2CH_2CH_2CH_2N$morpholine, DME;

c) CF_3CO_2H, CF_3CO_3H, $CHCl_3$.

Reaction of 4-amino-3-nitrophenol (**52**) with cyanamide and condensation under basic conditions gave amine **53** (Scheme 10). Alkylation of **53** with 2-bromoethylmethylether gave amine **54**. Diazotisation of amine **54** and chlorination of the intermediate phenol **55** gave chloride **56**. Stille reaction of chloride **56** with tetraethyltin and $Pd(PPh_3)_4$ in DMF gave the 1-oxide **57** which was oxidised to 1,4-dioxide **58**.

Scheme 10



Reagents:

a) NH_2CN, HCl; then NaOH;

b) $BrCH_2CH_2OMe$, K_2CO_3, DMF;

c) $NaNO_2$, aqueous HCl;

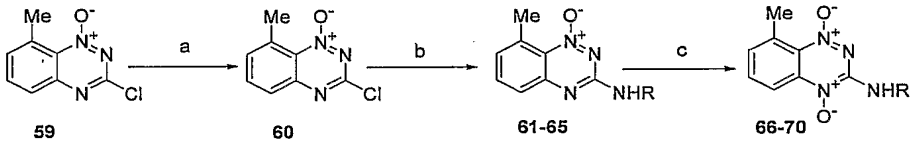
d) $POCl_3$;

e) Et_4Sn, $Pd(PPh_3)_4$, DMF;

f) CF_3CO_3H, $CHCl_3$.

Diazotization of amine **59** (Hay et. al., *J. Med. Chem.* **2003**, 46, 169) and chlorination of the intermediate alcohol gave chloride **60** (Scheme 11). Nucleophilic displacement of **60** with a variety of amines gave 1-oxides **61-65** which underwent selective aromatic *N*-oxidation under acidic conditions to give 1,4-dioxides **66-70**.

Scheme 11



Reagents:

a) $NaNO_2$, TFA, then $POCl_3$, DMF;

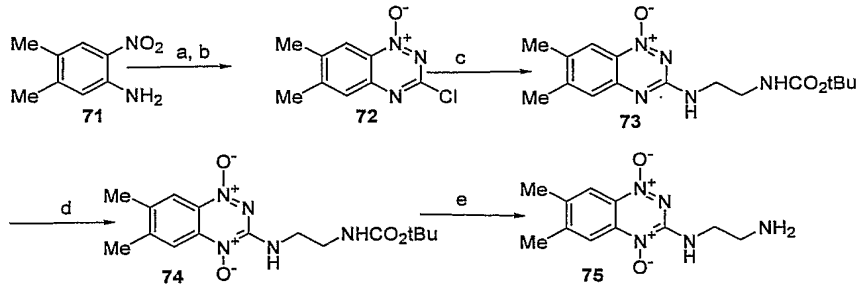
b) amine, DME;

c) CF_3CO_2H, CF_3CO_3H, DCM.

Reagent	1-oxide	1,4-dioxide	R =
$NH_2CH_2CH_2NMe_2$	**61**	**66**	$-CH_2CH_2NMe_2$
$NH_2CH_2CH_2NEt_2$	**62**	**67**	$-CH_2CH_2NEt_2$
$NH_2CH_2CH_2NPr_2$	**63**	**68**	$-CH_2CH_2NPr_2$
$NH_2CH_2CH_2N$piperidine	**64**	**69**	$-CH_2CH_2N$piperidine
$NH_2CH_2CH_2N$-2,6-Me_2piperidine	**65**	**70**	$-CH_2CH_2N$-2,6-Me_2piperidine

Reaction of nitroaniline **71** with cyanamide and condensation of the intermediate guanidine under basic conditions, followed by diazotisation and chlorination gave chloride **72** (Scheme 12). Displacement of **72** with *tert*-butyl 2-aminoethylcarbamate gave 1-oxide **73** which was oxidised to 1,4-dioxide **74** and deprotected to give **75**.

Scheme 12

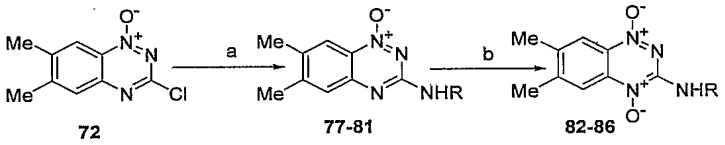


Reagents:

a) NH_2CN, HCl; then NaOH;
b) $NaNO_2$, TFA; then $POCl_3$, DMF;
c) $NH_2CH_2CH_2NHCO_2tBu$, DME;
d) MCPBA, DCM;
e) HCl, MeOH.

Nucleophilic displacement of chloride **72** with a variety of amines gave 1-oxides **77-81** which underwent selective aromatic *N*-oxidation under acidic conditions to give 1,4-dioxides **82-86** (Scheme 13).

Scheme 13



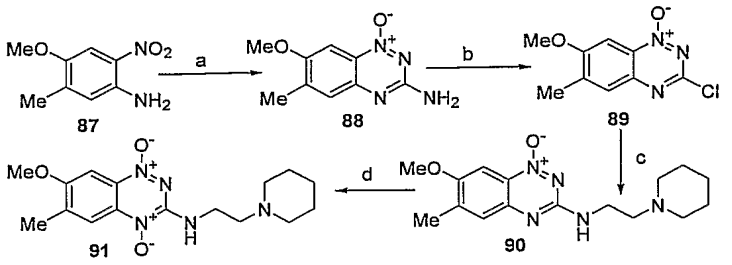
Reagents:

a) $NaNO_2$, TFA; then $POCl_3$, DMF;
b) amine, DME;
c) CF_3CO_3H, DCM.

Reagent	1-oxide	1,4-dioxide	R =
$NH_2CH_2CH_2NMe_2$	**77**	**82**	$-CH_2CH_2NMe_2$
$NH_2CH_2CH_2NEt_2$	**78**	**83**	$-CH_2CH_2NEt_2$
$NH_2CH_2CH_2NPr_2$	**79**	**84**	$-CH_2CH_2NPr_2$
$NH_2CH_2CH_2N$piperidine	**80**	**85**	$-CH_2CH_2N$piperidine
$NH_2CH_2CH_2CH_2N$morpholine	**81**	**86**	$-CH_2CH_2CH_2N$morpholine

Reaction of nitroaniline **87** [Arnold & McCool, *J. Am. Chem. Soc.* **1942**, *64*, 1315] with cyanamide and condensation of the intermediate guanidine under basic conditions gave amine **88** (Scheme 14). Diazotisation and chlorination of **88** gave chloride **89**. Displacement of **89** with 2-(1-piperidinyl)ethylamine gave 1-oxide **90**, which was oxidised to 1,4-dioxide **91**.

Scheme 14



Reagents:

a) NH_2CN, HCl; then NaOH;

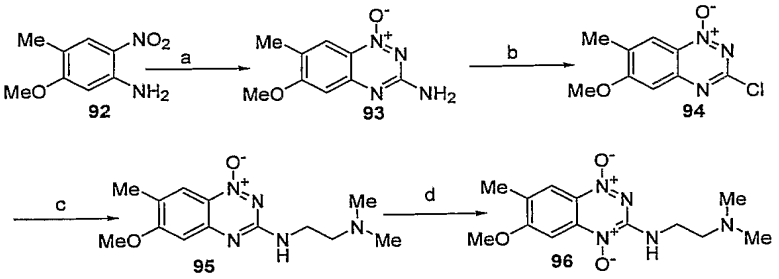
b) $NaNO_2$, TFA; then $POCl_3$, DMF;

c) $NH_2CH_2CH_2N$piperidine, DME;

d) CF_3CO_2H, CF_3CO_3H, $CHCl_3$.

Reaction of nitroaniline **92** with cyanamide and condensation of the intermediate guanidine under basic conditions gave amine **93** (Scheme 15). Diazotisation and chlorination of **93** gave chloride **94**. Displacement of **94** with *N,N*-dimethylethylenediamine gave 1-oxide **95** which was oxidised to 1,4-dioxide **96**.

Scheme 15



Reagents:

a) NH_2CN, HCl; then NaOH;

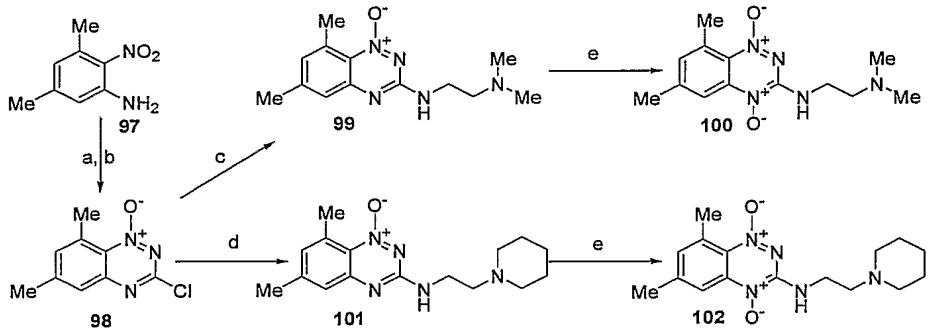
b) $NaNO_2$, TFA; then $POCl_3$, DMF;

c) $NH_2CH_2CH_2NMe_2$, DME;

d) CF_3CO_2H, CF_3CO_3H, $CHCl_3$.

Reaction of nitroaniline **97** with cyanamide and condensation of the intermediate guanidine under basic conditions, followed by diazotisation and chlorination gave chloride **98** (Scheme 16). Displacement of **98** with amines gave 1-oxides **99** and **101** which were oxidised with trifluoroperacetic acid to give 1,4-dioxides **100** and **102**, respectively.

Scheme 16



Reagents:

a) NH_2CN, HCl; then NaOH;

b) $NaNO_2$, TFA; then $POCl_3$, DMF;

c) $NH_2CH_2CH_2NMe_2$, DME;

d) $NH_2CH_2CH_2N$piperidine, DME;

e) CF_3CO_2H, CF_3CO_3H, $CHCl_3$.

Examples of the compounds of the invention

The following examples are representative of the invention and the detailed methods for preparing them, however, the scope of the invention is not to be taken as being limited to these examples.

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C spectra. Spectra were obtained in $CDCl_3$ unless otherwise specified, and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Assignments were determined using COSY, HSQC, and HMBC two-dimensional experiments. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High-resolution spectra were obtained at nominal resolutions of 3000, 5000, or 10000 as appropriate. All spectra were obtained as electron impact (EI) using PFK as the reference unless otherwise stated. Solutions in organic solvents were dried with anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}) with visualization of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel, (Merck 230-400 mesh). All compounds designated for biological testing were

analysed at >99% purity by reverse phase HPLC using a Philips PU4100 liquid chromatograph, a Phenomenex BondClone 10-C18 stainless steel column (300mm x 3.9 mm i.d.) and a Philips PU4120 diode array detector. Chromatograms were run using various gradients of aqueous (1 M NaH_2PO_4, 0.75 M heptanesulfonic acid, 0.5 M dibutylammonium phosphate, and MilliQ water in a 1:1:1:97 ratio) and organic (80% MeOH/MilliQ water) phases. DCM refers to dichloromethane; DME refers to 1,2-dimethoxyethane, DMF refers to dry dimethylformamide; ether refers to diethyl ether; EtOAc refers to ethyl acetate; EtOH refers to ethanol; MeOH refers to methanol; pet. ether refers to petroleum ether, boiling range 40-60 °C; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl. All solvents were freshly distilled.

Example 1

3-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]propanenitrile (3).

3-[(1-Oxido-1,2,4-benzotriazin-3-yl)amino]propanenitrile (2). A solution of chloride **1** [Robbins & Schofield, *J. Chem. Soc.* **1957**, 3186] (776 mg, 4.3 mmol), 3-aminopropanenitrile fumarate (2.74 g, 21.4 mmol) and Et_3N (3.6 mmol, 25.6 mmol) in DME (50 mL) was stirred at reflux temperature for 6 h. The solution was partitioned between DCM (100 mL) and water (100 mL), the aqueous fraction washed with DCM (2 × 50 mL), the combined organic fraction dried and the solvent evaporated. The residue was purified by chromatography, eluting with 10% EtOAc/DCM, to give nitrile **2** (771 mg, 84%) as yellow needles, mp (EtOAc/DCM) 191–193 °C; ^{1}H NMR δ 8.29 (dd, *J* = 8.7, 1.1 Hz, 1 H, H-8'), 7.76 (ddd, *J* = 8.5, 7.0, 1.1 Hz, 1 H, H-6'), 7.64 (dd, *J* = 8.5, 1.0 Hz, 1 H, H-5'), 7.37 (ddd, *J* = 8.7, 7.0, 1.0 Hz, 1 H, H-7'), 6.00 (br s, 1 H, NH), 3.87 (q, *J* = 6.5 Hz, 2 H, H-3), 2.85 (t, *J* = 6.5 Hz, 2 H, H-2); ^{13}C NMR δ 158.0, 148.4, 135.9, 131.3, 126.8, 126.7, 120.4, 117.9, 37.6, 18.1. Anal. calcd for $C_{10}H_9N_4O$: C, 55.8; H, 4.2; N, 32.6; found, C, 55.9; H, 4.3; N, 32.6%.

3-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]propanenitrile (3). A solution of MCPBA (1.45 g, 5.9 mmol) in DCM (25 mL) was added to a stirred solution of nitrile **2** (634 mg, 5.9 mmol) and the mixture stirred at 20 °C for 16 h. The mixture was diluted with $CHCl_3$ (100 mL), washed with dilute NH_4OH solution (2 × 50 mL) and water (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient 0–5% MeOH/20% EtOAc/DCM, to give (i) starting material **2** (280 mg, 44%) spectroscopically identical with the material prepared above; and (ii) 1,4-dioxide **3** (219 mg, 32%) as a red powder, mp (EtOAc/DCM) 204–206 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.57 (t, *J* = 6.2 Hz, 1 H, NH), 8.23 (d, *J* = 8.6 Hz, 1 H, H-8'), 8.17

(d, J = 8.5 Hz, 1 H, H-5'), 7.96 (ddd, J = 8.5, 7.3, 1.0 Hz, 1 H, H-6'), 7.61 (ddd, J = 8.6, 7.3, 0.9 Hz, 1 H, H-7'), 3.67 (dd, J = 6.5, 6.2 Hz, 2 H, H-3), 2.88 (t, J = 6.5 Hz, 2 H, H-2); ^{13}C NMR [$(CD_3)_2SO$] δ 149.5, 138.2, 135.5, 130.4, 127.3, 121.0, 119.0, 116.9, 36.7, 17.2. Anal. calcd for $C_{10}H_9N_5O_2$: C, 51.9; H, 3.9; N, 30.3; found, C, 52.0; H, 3.8; N, 30.4%.

Example 2

Ethyl [(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]acetate (5).

Ethyl [(1-Oxido-1,2,4-benzotriazin-3-yl)amino]acetate (4). A mixture of chloride **1** (2.02 g, 11.1 mmol), glycine ethyl ester hydrochloride (2.33 g, 16.7 mmol) and Et_3N (4.2 mL, 30 mmol) in DME (100 mL) was heated at reflux temperature for 6 h. The solvent was evaporated and the residue partitioned between DCM/water (200 mL), the aqueous fraction extracted with DCM (2 × 50 mL), the combined organic fraction dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 10% EtOAc/DCM, to give ester **4** (2.75 g, 99%) as a yellow solid, mp (EtOAc/DCM) 136–138 °C; ^{1}H NMR δ 8.27 (dd, J = 8.6, 1.0 Hz, 1 H, H-8), 7.72 (ddd, J = 8.5, 7.0, 1.4 Hz, 1 H, H-6), 7.62 (dd, J = 8.5, 1.0 Hz, 1 H, H-5), 7.34 (ddd, J = 8.6, 7.0, 1.0 Hz, 1 H, H-7), 5.87 (br s, 1 H, NH), 4.30 (d, J = 5.7 Hz, 2 H, CH_2N), 4.26 (q, J = 7.2 Hz, 2 H, CH_2O), 1.31 (t, J = 7.2 Hz, 3 H, CH_3); ^{13}C NMR δ 169.9, 158.4, 148.5, 135.6, 131.2, 126.7, 125.5, 120.4, 61.6, 43.2, 14.2. Anal. calcd for $C_{11}H_{12}N_4O_3$: C, 53.2; H, 4.9; N, 22.6; found, C, 53.4; H, 5.0; N, 22.6%.

Ethyl [(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]acetate (5). A solution of MCPBA (2.38 g, 9.7 mmol) in DCM (20 mL) was added dropwise to a stirred solution of 1-oxide **4** (1.20 g, 4.8 mmol) in DCM (50 mL) and stirred at 20 °C for 3 h. The mixture was diluted with DCM (100 mL), washed with dilute NH_4OH solution (100 mL) and water (100 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20–40%) of EtOAc/DCM, then a gradient (0–5%) of MeOH/DCM, to give (i) starting material **4** (0.70 g, 58%), spectroscopically identical with the material prepared above; and (ii) 1,4-dioxide **5** (234 mg, 18%) as yellow solid, mp (EtOAc/pet ether)156–158 °C, ^{1}H NMR δ 8.34 (d, J = 8.7 Hz, 2 H, H-5, H-8), 7.91 (br s, 1 H, NH), 7.50–7.61 (m, 2 H, H-6, H-7), 4.37 (br s, 2 H, CH_2), 4.26 (q, J = 7.1 Hz, 2 H, CH_2), 1.30 (t, J = 7.1 Hz, 3 H, CH_3); ^{13}C NMR δ 168.5, 149.7, 138.4, 136.0, 131.0, 129.6, 121.7, 117.6, 61.9, 42.8, 14.1; MS (EI^+) *m/z* 264 (M^+, 30%), 284 (40), 175 (100); HRMS (EI^+) calcd for $C_{11}H_{12}N_4O_4$ (M^+) *m/z* 264.0859,

found 264.0852. Anal. calcd for $C_{11}H_{12}N_4O_4$: C, 50.0; H, 4.6; N, 21.2; found C, 50.8; H, 4.5; N, 21.1%.

Example 3

3-Ethyl-6-methyl-1,2,4-benzotriazine 1,4-Dioxide (9).

3-Chloro-6-methyl-1,2,4-benzotriazine 1-Oxide (7). Sodium nitrite (7.09 g, 103 mmol) was added in small portions to a stirred solution of 6-methyl-1,2,4-benzotriazin-3-amine 1-oxide (**6**) [Hay et. al., *J. Med. Chem.* **2003**, 46, 169] (9.05 g, 51.4 mmol) in trifluoroacetic acid (80 mL) at 5 °C and the solution stirred at 20 °C for 3 h. The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (100 mL) and DMF (0.5 mL) and stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water, stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **7** (7.86 g, 78%) as a pale yellow solid, mp (EtOAc/DCM) 156–158 °C; ^{1}H NMR δ 8.29 (d, J = 8.8 Hz, 1 H, H-8), 7.74 (d, J = 1.7 Hz, 1 H, H-5), 7.56 (dd, J = 8.8, 1.7 Hz, 1 H, H-7), 2.61 (s, 3 H, CH_3). Anal. calcd for $C_8H_6ClN_3O$: C, 49.1; H, 3.1; N, 21.5; found C, 49.2; H, 3.4; N, 21.5%.

3-Ethyl-6-methyl-1,2,4-benzotriazine 1-Oxide (8). $Pd(PPh_3)_4$ (410 mg, 0.35 mmol) was added to a stirred solution of chloride **7** (728 mg, 3.6 mmol) and tetraethyltin (1.4 mL, 7.1 mmol), the solution degassed, and stirred under N_2 at reflux temperature for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/pet. ether to give an oil which was further purified by chromatography, eluting with 5% EtOAc/DCM, to give (i) starting material **7** (412 mg, 56%) and (ii) 1-oxide **8** (250 mg, 37%) as a white solid, mp (EtOAc/DCM) 68–70 °C; ^{1}H NMR δ 8.33 (d, J = 8.8 Hz, 1 H, H-8), 7.74 (br s, 1 H, H-5), 7.49 (dd, J = 8.8, 1.7 Hz, 1 H, H-7), 3.02 (q, J = 7.6 Hz, 2 H, CH_2), 2.59 (s, 3 H, CH_3), 1.44 (t, J = 7.6 Hz, 3 H, CH_3); ^{13}C NMR δ 168.2, 147.8, 147.1, 132.0, 131.6, 127.5, 119.8, 30.7, 22.1, 12.2. Anal. calcd for $C_{10}H_{11}N_3O$: C, 63.5; H, 5.9; N, 22.2; found C, 63.5; H, 6.0; N, 22.3%.

3-Ethyl-6-methyl-1,2,4-benzotriazine 1,4-Dioxide (9). Hydrogen peroxide (70%; 0.55 ml, ca. 10.9 mol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.55 mL, 10.9 mmol) in DCM (15 mL) at 5 °C. The solution was stirred at 20 °C for 5 min then cooled to 5 °C, added to a solution of 1-oxide **8** (207 mg, 1.1

mmol) and the mixture stirred vigorously for 16 h. Dilute aqueous NH_3 solution was added and the mixture stirred at 20 °C for 30 minutes. The mixture was extracted with $CHCl_3$ (5 × 30 mL), the combined organic fraction dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give 1,4-dioxide **9** (70 mg, 31%) as pale yellow powder, mp (EtOAc/pet. ether) 160–163 °C; ^{1}H NMR δ 8.35 (d, J = 8.8 Hz, 1 H, H-8), 8.30 (d, J = 1.7 Hz, 1 H, H-5), 7.64 (dd, J = 8.8, 1.7 Hz, 1 H, H-7), 3.21 (q, J = 7.5, Hz, 2 H, CH_2), 2.65 (s, 3 H, CH_3), 1.44 (t, J = 7.5 Hz, 3 H, CH_3); ^{13}C NMR δ 156.6, 147.7, 139.5, 133.6, 132.9, 121.4, 118.4, 24.0, 22.3, 9.2. Anal. calcd for $C_{10}H_{11}N_3O_2$: C, 58.2; H, 5.4; N, 20.5; found C, 58.6; H, 5.3; N, 20.6%.

Example 4

2-(6-Methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)ethanol (12).

3-Allyl-6-methyl-1,2,4-benzotriazine 1-Oxide (10). $Pd(PPh_3)_4$ (370 mg, 0.32 mmol) was added to a stirred solution of chloride **6** (1.24 g, 6.3 mmol) and allyltributyltin (2.2 mL, 7.0 mmol), the solution degassed, and stirred under N_2 at reflux temperature for 6 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/pet. ether, to give an oil which was further purified by chromatography, eluting with 5% EtOAc/DCM, to give alkene **10** (0.97 g, 74%) as a white solid, mp (EtOAc/pet. ether) 65–67 °C, ^{1}H NMR δ 8.32 (d, J = 8.8 Hz, 1 H, H-8), 7.76 (d, J = 1.7 Hz, 1 H, H-5), 7.50 (dd, J = 8.8, 1.7 Hz, 1 H, H-7), 6.13–6.21 (m, 1 H, H-2'), 5.30 (dq, J = 17.0, 1.5 Hz, 1 H, H-3'), 5.22 (dq, J = 10.1, 1.5 Hz, 1 H, H-3'), 3.76 (dq, J = 6.9, 1.5 Hz, 2 H, H-1'), 2.58 (s, 3 H, CH_3); ^{13}C NMR δ 165.3, 147.8, 147.3, 132.8, 132.5, 131.6, 127.6, 119.7, 118.4, 41.8, 22.1. Anal. calcd for $C_{11}H_{11}N_3O$: C, 65.7; H, 5.5; N, 20.9; found C, 65.8; H, 5.5; N, 21.0%.

2-(6-Methyl-1-oxido-1,2,4-benzotriazin-3-yl)ethanol (11). Ozone was bubbled into a solution of alkene **10** (1.12 g, 5.6 mmol) in DCM/MeOH (1:1, 80 mL) at –78 °C until a blue colour persisted. The solution was purged with N_2 to remove excess ozone, then a solution of $NaBH_4$ (210 mg, 5.6 mmol) in EtOH (10 mL) added dropwise and the solution allowed to warm to 20 °C over 1 h. HOAc (2 mL) was added and the solution stirred at 20 °C for 30 min. The solvent was evaporated and the residue partitioned between DCM (50 mL) and water (3 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50–100%) of EtOAc/pet. ether, to give alcohol **11** (780 mg, 68%) as pale yellow prisms, mp (EtOAc/pet. ether) 121–123 °C; ^{1}H NMR δ 8.32 (d, J

= 8.8 Hz, 1 H, H-8), 7.74 (d, J = 1.7 Hz, 1 H, H-5), 7.50 (dd, J = 8.8, 1.7 Hz, 1 H, H-7), 4.04–4.10 (m, 2 H, CH_2O), 3.27 (t, J = 5.6 Hz, 2 H, CH_2), 3.17 (t, J = 6.3 Hz, 1 H, OH), 2.60 (s, 3 H, CH_3); ^{13}C NMR δ 165.5, 147.6, 147.3, 132.4, 131.9, 127.4, 119.8, 60.1, 39.0, 22.1. Anal. calcd for $C_{10}H_{11}N_3O_2$: C, 58.5; H, 5.4; N, 20.5; found C, 58.8; H, 5.5; N, 20.5%.

2-(6-Methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)ethanol (12). Trifluoroacetic anhydride (248 μL, 1.75 mmol), was added dropwise to a solution of 1-oxide **11** (300 mg, 1.46 mmol) and pyridine (142 μL, 1.75 mmol) in DCM (20 mL) and stirred at 20 °C for 16 h. The solution was partitioned between DCM (80 mL) and water (80 mL), the organic fraction dried and the solvent evaporated. The residue was dissolved in $CHCl_3$ (30 mL). Hydrogen peroxide (70%; 0.73 ml, ca. 14.6 mol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.06 mL, 14.6 mmol) in DCM (15 mL) at 5 °C. The solution was stirred at 20 °C for 5 min then cooled to 5 °C, added to the solution of trifluoroacetate and the mixture stirred vigorously for 16 h. Dilute aqueous NH_3 solution was added and the mixture stirred at 20 °C for 30 minutes. The mixture was extracted with $CHCl_3$ (5 × 30 mL), the combined organic fraction dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give 1,4-dioxide **12** (183 mg, 57%) as pale yellow needles, mp (EtOAc/pet. ether) 168–171 °C; ^1H NMR δ 8.26 (d, J = 8.8 Hz, 1 H, H-8), 8.18 (d, J = 1.7 Hz, 1 H, H-5), 7.78 (dd, J = 8.8, 1.7 Hz, 1 H, H-7), 4.74 (t, J = 6.0 Hz, 1 H, OH), 3.87 (dd, J = 6.5, 6.0 Hz, 2 H, CH_2O), 3.19 (t, J = 6.5 Hz, 2 H, CH_2), 2.61 (s, 3 H, CH_3); ^{13}C NMR δ 152.8, 147.1, 139.0, 133.6, 132.7, 120.7, 117.5, 56.3, 33.7, 21.5.; Anal. calcd. for $C_{10}H_{11}N_3O_3$: C, 54.3; H, 5.0; N, 19.0; found C, 54.5; H, 5.0; N, 19.1%.

Example 5

3-(2-Methoxyethyl)-6-methyl-1,2,4-benzotriazine 1,4-Dioxide (14).

3-(2-Methoxyethyl)-6-methyl-1,2,4-benzotriazine 1-Oxide (13). Three aliquots of $TMSCH_2N_2$ (1.1 mL, 2.1 mmol) were added to a stirred solution of alcohol **11** (433 mg, 2.1 mmol) and HBF_4 (0.26 mL, 2.1 mmol) in DCM (30 mL) over 3 h. The solution was stirred at 20 °C for 16 h, the solvent evaporated and the residue purified by chromatography, eluting with 50% EtOAc/pet. ether, to give (i) methyl ether **13** (119 mg, 19%) as a yellow powder, mp (EtOAC/pet. ether) 77–79 °C; ^1H NMR δ 8.32 (d, J = 8.8 Hz, 1 H, H-8), 7.56 (d, J = 1.7 Hz, 1 H, H-5), 7.50 (dd, J = 8.8, 1.7 Hz, 1 H, H-6), 3.95 (t, J = 6.5 Hz, 2 H, CH_2O), 3.37 (s, 3 H, OCH_3), 3.27 (t, J = 6.5 Hz, 2 H, CH_2),

2.58 (s, 3 H, CH_3); ^{13}C NMR δ 164.7, 147.7, 147.2, 132.2, 131.8, 127.6, 119.7, 70.1, 58.7, 37.6, 22.1; and (ii) starting material **11** (360 mg, 62%), spectroscopically identical to sample prepared above.

3-(2-Methoxyethyl)-6-methyl-1,2,4-benzotriazine 1,4-Dioxide (14). Hydrogen peroxide (270 μL, 5.4 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (0.77 mL, 5.4 mmol) in DCM (5 mL) at 5 °C and the solution stirred at 5 °C for 5 min, stirred at 20 °C for 10 min, cooled to 5 °C and added to a stirred solution of 1-oxide **13** (119 mg, 0.54 mmol) in $CHCl_3$ (10 mL) at 5 °C. The solution was stirred at 20 °C for 24 h, diluted with dilute aqueous NH_3 (50 mL) and extracted with $CHCl_3$ (3 × 20 mL). The organic fraction was dried and the solvent evaporated (CAUTION: use blast shield). The residue was purified by chromatography, eluting with 20% EtOAc/DCM, to give 1,4-dioxide **14** (60 mg, 47%) as a yellow powder, mp 154–158 °C; ^1H NMR δ 8.36 (d, J = 8.9 Hz, 1 H, H-8), 8.31 (d, J = 1.6 Hz, 1 H, H-5), 7.65 (dd, J = 8.9, 1.6 Hz, 1 H, H-7), 3.97 (t, J = 6.4 Hz, 2 H, CH_2O), 3.48 (t, J = 6.4 Hz, 2 H, CH_2), 3.40 (s, 3 H, OCH_3), 2.66 (s, 3 H, CH_3); ^{13}C NMR δ 153.3, 147.8, 139.5, 133.7, 133.1, 121.4, 118.5, 67.3, 58.7, 30.7, 22.3. Anal. calcd for $C_{11}H_{13}N_3O_3$: C, 56.2; H, 5.6; N, 17.9; found C, 56.4; H, 5.9; N, 16.9%.

Example 6

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (18).**

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-methyl-1-oxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (13).** *N*,*N*-Dimethylethanediamine (705 μL, 6.6 mmol) was added to a stirred solution of chloride **6** (518 mg, 2.7 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **13** (603 mg, 92%) as a yellow solid, mp (MeOH/EtOAc) 143–145 °C; ^1H NMR δ 8.11 (d, J = 8.8 Hz, 1 H, H-8), 7.35 (d, J = 1.7 Hz, 1 H, H-5), 7.07 (dd, J = 8.8, 1.7 Hz, 1 H, H-7), 5.89 (br s, 1 H, NH), 3.50–3.56 (m, 2 H, CH_2N), 2.52–2.56 (m, 2 H, CH_2N), 2.45 (s, 3 H, CH_3), 2.26 [s, 6 H, $N(CH_3)_2$]; ^{13}C NMR δ 159.2, 149.1, 146.9, 129.2, 126.9, 125.3, 120.1, 57.5, 45.1 (2), 38.7, 22.0. Anal. calcd for $C_{12}H_{17}N_5O$: C, 58.3; H, 6.9; N, 28.3; found C, 58.5: H, 7.1; N, 28.6%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (18).** Hydrogen peroxide (70%, 1.1 mL, ca. 22.9 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (3.2 mL, 22.9 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **13** (566 mg, 2.3 mmol) and trifluoroacetic acid (353 μL, 4.6 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **18** (207 mg, 34%) as a red solid, mp (MeOH/EtOAc) 187–189 °C; ^{1}H NMR δ 8.19 (d, *J* = 9.0 Hz, 1 H, H-8), 8.05 (d, *J* = 1.7 Hz, 1 H, H-5), 7.44 (br s, 1 H, NH), 7.29 (dd, *J* = 9.0, 1.7 Hz, 1 H, H-7), 3.58–3.64 (m, 2 H, CH_2N), 2.57–2.61 (m, 2 H, CH_2N), 2.56 (s, 3 H, CH_3), 2.28 [s, 6 H, $(CH_3)_2$]; ^{13}C NMR δ 149.9, 148.0, 138.2, 129.3, 128.8, 121.4, 116.0, 57.4, 45.2 (2), 38.8, 22.3. Anal. calcd for $C_{12}H_{17}N_5O_2$: C, 54.7; H, 6.5; N, 26.6; found C, 54.3: H, 6.7; N, 26.8%.

Example 7

***N*^{1},*N*^{1}-Diethyl-*N*^{2}-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (19).**

***N*^{1},*N*^{1}-Diethyl-*N*^{2}-(6-methyl-1-oxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (14).** *N*,*N*-Diethylethanediamine (0.68 mL, 4.9 mmol) was added to a stirred solution of chloride **6** (380 mg, 1.9 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) MeOH/DCM, to give 1-oxide **14** (502 mg, 94%) as a yellow solid, mp (MeOH/EtOAc) 78–80 °C; ^{1}H NMR δ 8.13 (d, *J* = 8.7 Hz, 1 H, H-8), 7.36 (br s, 1 H, H-5), 7.08 (dd, *J* = 8.7, 1.6 Hz, 1 H, H-7), 5.98 (br s, 1 H, NH), 3.50–3.55 (m, 2 H, CH_2N), 2.60–2.73 (m, 2 H, CH_2N), 2.59 (q, *J* = 7.1 Hz, 4 H, 2 × CH_2N), 2.46 (s, 3 H, CH_3), 1.05 (t, *J* = 7.1 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 159.1, 149.1, 146.9, 129.1, 126.9, 125.3, 120.1, 51.2, 46.7 (2), 38.7, 22.0, 11.7 (2). Anal. calcd for $C_{14}H_{21}N_5O$: C, 61.1; H, 7.7; N, 25.4; found C, 60.8: H, 8.0; N, 25.2%.

***N*^{1},*N*^{1}-Diethyl-*N*^{2}-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (19).** Hydrogen peroxide (70%, 0.66 mL, ca. 13.1 mmol) was added

dropwise to a stirred solution of trifluoroacetic anhydride (1.9 mL, 13.1 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **14** (362 mg, 1.3 mmol) and trifluoroacetic acid (203 μL, 2.6 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **19** (168 mg, 44%) as a red solid, mp (MeOH/EtOAc) 168–171 °C; ^{1}H NMR δ 8.19 (d, J = 9.0 Hz, 1 H, H-8), 8.06 (br s, 1 H, H-5), 7.52 (br s, 1 H, NH), 7.27 (dd, J = 9.0, 1.6 Hz, 1 H, H-7), 3.57–3.61 (m, 2 H, CH_2N), 2.74 (dd, J = 6.2, 6.0 Hz, 2 H, CH_2N), 2.59 (q, J = 7.1 Hz, 4 H, 2 × CH_2N), 2.56 (s, 3 H, CH_3), 1.05 (t, J = 7.1 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 150.0, 147.9, 138.2, 129.2, 128.8, 121.4, 116.1, 51.3, 46.8 (2), 39.1, 22.3, 11.9 (2). Anal. calcd for $C_{14}H_{21}N_5O_2 \cdot ¼H_2O$: C, 56.8; H, 7.3; N, 23.7; found C, 57.0; H, 7.3; N, 23.8%.

Example 8

N^1-(6-Methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dipropyl-1,2-ethanediamine (20).

N^1-(6-Methyl-1-oxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dipropyl-1,2-ethanediamine (15). *N*,*N*-dipropyl-1,2-ethanediamine (0.83 mL, 5.7 mmol) was added to a stirred solution of 3-chloride **6** (448 mg, 2.3 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 3 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–6%) of MeOH/DCM, to give 1-oxide **15** (697 mg, 100%) as a yellow powder, mp (EtOAc/pet ether) 88–90 °C, ^{1}H NMR δ 8.13 (d, J = 8.8 Hz, 1 H, H-8), 7.36 (br s, 1 H, H-5), 7.08 (dd, J = 8.8, 1.8 Hz, 1 H, H-7), 5.89 (br s, 1 H, NH), 3.48–3.52 (m, 2 H, CH_2N), 2.66–2.69 (m, 2 H, CH_2N), 2.46 (s, 3 H, CH_3), 2.39–2.43 (m, 4 H, 2 × CH_2N), 1.41–1.50 (m, 4 H, 2 × CH_2), 0.88 (t, J = 7.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 159.2, 149.1, 146.8, 129.1, 126.9, 125.3, 120.2, 55.9 (2), 52.5, 38.9, 22.0, 20.3 (2), 11.9 (2). Anal. calcd for $C_{16}H_{25}N_5O$: C, 63.3; H, 8.3; N, 23.0; found C, 63.3; H, 8.6; N, 23.3%.

N^1-(6-Methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dipropyl-1,2-ethanediamine (20). Hydrogen peroxide (ca. 70%, 0.63 mL, 12.5 mmol) was added

dropwise to a stirred solution of trifluoroacetic anhydride (1.8 mL, 12.5 mmol) in DCM (10 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **15** (379 mg, 1.3 mmol) and trifluoroacetic acid (192 μL, 2.5 mmol) in $CHCl_3$ (20 mL) at 5 °C. The solution was stirred at 5 °C for 16 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (3 × 30 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **20** (287 mg, 72%) as a red solid, mp (MeOH/EtOAc) 162–166 °C; ^{1}H NMR δ 8.20 (d, J = 9.0 Hz, 1 H, H-8), 8.07 (br s, 1 H, H-5), 7.58 (br s, 1 H, NH), 7.29 (dd, J = 9.0, 1.6 Hz, 1 H, H-7), 3.57–3.64 (m, 2 H, CH_2N), 2.73–2.79 (m, 2 H, CH_2N), 2.55 (s, 3 H, CH_3), 2.44–2.51 (m, 4 H, 2 × CH_2N), 1.47–1.56 (m, 4 H, 2 × CH_2), 0.92 (t, J = 7.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 149.9, 147.9, 138.3, 129.2, 128.8, 121.4, 116.1, 55.9 (2), 52.4, 38.9, 22.3, 20.2 (2), 11.8 (2). Anal. calcd for $C_{16}H_{25}N_5O_2 \cdot ½H_2O$: C, 58.5; H, 8.0; N, 21.3; found C, 58.6; H, 7.7; N, 21.5%.

Example 9

6-Methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (21). **6-Methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1-Oxide (16).** 2-(1-Piperidinyl)ethylamine (0.87 mL, 6.1 mmol) was added to a stirred solution of chloride **6** (476 mg, 2.4 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **16** (656 mg, 94%) as a yellow powder, mp (MeOH/EtOAc) 156–158 °C; ^{1}H NMR δ 8.13 (d, J = 8.7 Hz, 1 H, H-8), 7.36 (d, J = 1.7 Hz, 1 H, H-5), 7.08 (dd, J = 8.7, 1.7 Hz, 1 H, H-7), 5.98 (br s, 1 H, NH), 3.51–3.56 (m, 2 H, CH_2N), 2.54–2.58 (m, 2 H, CH_2N), 2.47 (s, 3 H, CH_3), 2.39–2.45 (m, 4 H, 2 × CH_2N), 1.55–1.61 (m, 4 H, 2 × CH_2), 1.42–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 159.1, 149.1, 146.9, 129.1, 126.9, 125.3, 120.1, 56.9, 54.3 (2), 37.9, 26.0 (2), 24.4, 22.0. Anal. calcd for $C_{15}H_{21}N_5O$: C, 62.7; H, 7.4; N, 24.4; found C, 62.8; H, 7.7; N, 24.5%.

6-Methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (21). Hydrogen peroxide (70%; 0.85 mL, ca. 16.8 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.4 mL, 16.8 mmol) in DCM (20 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5

°C and added to a stirred solution of 1-oxide **16** (483 mg, 1.7 mmol) and trifluoroacetic acid (256 μL, 3.4 mmol) in $CHCl_3$ (20 mL) at 5 °C. The solution was stirred at 5 °C for 16 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **21** (391 mg, 76%) as a red solid, mp (MeOH/EtOAc) 162–165 °C; ^{1}H NMR δ 8.20 (d, J = 9.0 Hz, 1 H, H-8), 8.07 (d, J = 1.6 Hz, 1 H, H-7), 7.53 (br s, 1 H, NH), 7.29 (dd, J = 9.0, 1.6 Hz, 1 H, H-5), 3.61–3.66 (m, 2 H, CH_2N), 2.62–2.66 (m, 2 H, CH_2N), 2.57 (s, 3 H, CH_3), 2.43–2.48 (m, 4 H, 2 × CH_2N), 1.58–1.64 (m, 4 H, 2 × CH_2), 1.42–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 150.3, 148.0, 138.3, 129.2, 128.4, 121.4, 116.1, 56.8, 54.4 (2), 38.2, 25.9 (2), 24.3, 22.3. Anal. calcd for $C_{15}H_{21}N_5O_2$: C, 59.4; H, 7.0; N, 23.1; found C, 59.1; H, 6.7; N, 22.9%.

Example 10

***N*-[2-(2,6-Dimethyl-1-piperidinyl)ethyl]-6-methyl-1,2,4-benzotriazin-3-amine 1,4-Dioxide (22).**

***N*-[2-(2,6-Dimethyl-1-piperidinyl)ethyl]- 6-methyl-1,2,4-benzotriazin-3-amine 1-Oxide (17).** 2-(2,6-Dimethyl-1-piperidinyl)ethylamine (834 mg, 5.3 mmol) was added to a stirred solution of chloride **6** (418 mg, 2.1 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) MeOH/DCM, to give 1-oxide **17** (597mg, 93%) as a yellow solid, mp (MeOH/EtOAc) 162–165 °C; ^{1}H NMR δ 8.12 (d, J = 8.9 Hz, 1 H, H-8), 7.35 (d, J = 1.7 Hz, 1 H, H-5), 7.09 (dd, J = 8.9, 1.7 Hz, 1 H, H-7), 5.57 (br s, 1 H, NH), 3.50–3.56 (m, 2 H, CH_2N), 2.87–2.91 (m, 2 H, CH_2N), 2.49–2.57 (m, 2 H, 2 × CH), 2.47 (s, 3 H, CH_3), 1.65–1.69 (m, 1 H, CH_2), 1.53–1.58 (m, 2 H, CH_2), 1.25–1.41 (m, 3 H, CH_2), 1.19 (d, J = 6.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 159.2, 149.1, 146.9, 129.2, 127.0, 125.4, 120.8, 57.3 (2), 47.4, 39.5, 34.2, 24.4 (2), 22.0 (2), 21.6. Anal. calcd for $C_{17}H_{25}N_5O$: C, 64.7; H, 8.0; N, 22.2; found C, 64.3: H, 7.3; N, 22.0%.

***N*-[2-(2,6-Dimethyl-1-piperidinyl)ethyl]-6-methyl-1,2,4-benzotriazin-3-amine 1,4-Dioxide Hydrochloride (22).** Hydrogen peroxide (70%, 0.75 mL, ca. 15.1 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.1 mL, 15.1 mmol)

in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **17** (455 mg, 1.5 mmol) and trifluoroacetic acid (233 μL, 3.0 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–8%) of MeOH/DCM, to give 1,4-dioxide **22** (146 mg, 29%) as a red solid, mp (MeOH/EtOAc) 171–174 °C; ^{1}H NMR δ 8.19 (d, J = 8.9 Hz, 1 H, H-8), 8.06 (d, J = 1.7 Hz, 1 H, H-5), 7.27–7.33 (m, 2 H, H-6, NH), 3.57–3.63 (m, 2 H, CH_2N), 2.90–2.95 (m, 2 H, CH_2N), 2.57 (s, 3 H, CH_3), 2.47–2.56 (m, 2 H, 2 × CH), 1.63–1.69 (m, 1 H, CH_2), 1.52–1.57 (m, 2 H, CH_2), 1.24–1.40 (m, 3 H, CH_2), 1.17 (d, J = 6.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 150.3, 148.0, 136.2, 129.2, 128.9, 121.4, 116.1, 57.3 (2), 47.3, 39.7, 34.1, 24.4 (2), 22.3 (2), 21.7. Anal. calcd for $C_{17}H_{25}N_5O_2$: C, 61.6; H, 7.6; N, 21.1; found C, 61.3: H, 7.6; N, 21.1%.

Example 11

***N*^{1}-(6-Isopropyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (27).**

6-Isopropyl-1,2,4-benzotriazin-3-amine 1-Oxide (24). A mixture of 5-isopropyl-2-nitroaniline (**23**) [Prasad, J. V. N. V. *Org. Lett.* **2000**, *2*, 1069] (4.53 g, 25.1 mmol) and cyanamide (4.23 g, 100 mmol) were mixed together at 100 °C, cooled to 50 °C, cHCl (15 mL) added carefully and the mixture heated at 100 °C for 4 h. The mixture was cooled to 50 °C, 7.5 M NaOH solution added until the mixture was strongly basic and the mixture stirred at 100 °C for 3 h. The mixture was cooled, diluted with water (200 mL), filtered, washed with water (3 × 50 mL), washed with ether (3 × 30 mL) and dried. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **24** (2.64 g, 52%) as a yellow powder, mp ($CHCl_3$) 220–222 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.04 (d, J = 8.9 Hz, 1 H, H-8), 7.32 (d, J = 1.8 Hz, 1 H, H-5), 7.27 (dd, J = 8.9, 1.8 Hz, 1 H, H-7), 7.24 (br s, 2 H, NH_2), 3.01 (sept, J = 6.9 Hz, 1 H, CH), 1.24 (d, J = 6.9 Hz, 6 H, 2 × CH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.3, 156.7, 149.0, 128.3, 124.2, 121.9, 119.7, 33.5, 22.9 (2).

3-Chloro-6-isopropyl-1,2,4-benzotriazine 1-Oxide (25). Sodium nitrite (1.71 g, 12.4 mmol) was added in small portions to a stirred solution of amine **24** (2.53 g, 12.4 mmol) in trifluoroacetic acid (80 mL) at 5 °C and the solution stirred at 20 °C for 3 h.

The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (70 mL) and DMF (0.5 mL) and stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water, stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **25** (1.74 g, 63%) as a pale yellow solid, mp (EtOAc/DCM) 81–83 °C; ^{1}H NMR δ 8.32 (d, *J* = 8.9 Hz, 1 H, H-8), 7.78 (d, *J* = 1.8 Hz, 1 H, H-5), 7.63 (dd, *J* = 8.9, 1.8 Hz, 1 H, H-7), 3.15 (sept, *J* = 6.9 Hz, 1 H, CH), 1.35 (d, *J* = 6.9 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 159.4, 157.0, 147.8, 132.2, 131.0, 124.6, 120.1, 34.6, 23.2 (2). Anal. calcd for $C_{10}H_{10}ClN_3O$: C, 53.7; H, 4.5; N, 18.8; found C, 53.7; H, 4.4; N, 19.0%.

N^1-(6-Isopropyl-1-oxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dimethyl-1,2-ethanediamine (26). *N*,*N*-Dimethylethanediamine (0.37 mL, 3.4 mmol) was added to a stirred solution of chloride **25** (300 mg, 1.3 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **26** (348 mg, 94%) as a yellow solid, mp (MeOH/EtOAc) 87–89 °C; ^{1}H NMR δ 8.16 (d, *J* = 8.9 Hz, 1 H, H-8), 7.40 (br s, 1 H, H-5), 7.16 (dd, *J* = 8.9, 1.7 Hz, 1 H, H-7), 5.91 (br s, 1 H, NH), 3.52–3.56 (m, 2 H, CH_2N), 3.00 (sept, *J* = 6.9 Hz, 1 H, CH), 2.54–2.58 (m, 2 H, CH_2N), 2.27 [s, 6 H, $N(CH_3)_2$], 1.29 (d, *J* = 6.9 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 159.2, 157.5, 149.3, 129.3, 124.8, 122.6, 120.3, 57.5, 45.1 (2), 38.7, 34.5, 22.3 (2). Anal. calcd for $C_{14}H_{21}N_5O \cdot ¼CH_3OH$: C, 60.4; H, 7.8; N, 24.7; found C, 60.5: H, 7.9; N, 25.0%.

N^1-(6-Isopropyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dimethyl-1,2-ethanediamine (27). Hydrogen peroxide (70%, 0.47 mL, ca. 9.3 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.3 mL, 9.3 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **26** (257 mg, 0.9 mmol) and trifluoroacetic acid (144 μL, 1.9 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 20 mL). The organic fraction was dried and the solvent evaporated. The residue was

purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **27** (187 mg, 69%) as a red solid, mp (MeOH/EtOAc) 171–174 °C; ^{1}H NMR δ 8.23 (d, *J* = 9.0 Hz, 1 H, H-8), 8.09 (d, *J* = 1.8 Hz, 1 H, H-5), 7.47 (br s, 1 H, NH), 7.36 (dd, *J* = 9.0, 1.8 Hz, 1 H, H-7), 3.60–3.65 (m, 2 H, CH_2N), 3.11 (sept, *J* = 6.9 Hz, 1 H, CH), 2.60 (dd, *J* = 6.1, 6.0 Hz, 2 H, CH_2N), 2.29 [s, 6 H, $(CH_3)_2$], 1.33 (d, *J* = 6.9 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 158.6, 149.9, 138.4, 128.9, 127.2, 121.5, 113.5, 57.4, 45.2 (2), 38.8, 34.9, 23.2 (2). Anal. calcd for $C_{14}H_{21}N_5O_2 \cdot ½H_2O$: C, 56.0; H, 7.4; N, 23.3; found C, 56.4; H, 7.1; N, 23.0%.

Example12

6-Isopropyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (29).

6-Isopropyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1-Oxide (28). 2-(1-Piperidinyl)ethylamine (0.47 mL, 3.4 mmol) was added to a stirred solution of chloride **25** (297 mg, 1.3 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **28** (394 mg, 94%) as a yellow solid, mp (MeOH/EtOAc) 124–127 °C; ^{1}H NMR δ 8.16 (d, *J* = 8.9 Hz, 1 H, H-8), 7.41 (br s, 1 H, H-5), 7.15 (dd, *J* = 8.9, 1.8 Hz, 1 H, H-7), 5.79 (br s, 1 H, NH), 3.51–3.55 (m, 2 H, CH_2N), 3.00 (sept, *J* = 6.9 Hz, 1 H, CH), 2.56 (dd, *J* = 6.1, 5.9 Hz, 2 H, CH_2N), 2.39–2.44 (m, 4 H, 2 × CH_2), 1.54–1.60 (m, 4 H, 2 × CH_2), 1.41–1.47 (m, 2 H, CH_2), 1.30 (d, *J* = 6.9 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 159.1, 157.5, 149.3, 129.3, 124.8, 122.6, 120.3, 56.8, 54.3 (2), 37.9, 34.5, 26.0 (2), 24.4, 23.3 (2). Anal. calcd for $C_{17}H_{25}N_5O$: C, 64.7; H, 8.0; N, 22.2; found C, 64.5: H, 8.3; N, 22.4%.

6-Isopropyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (29). Hydrogen peroxide (70%, 0.51 mL, ca. 10.1 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.4 mL, 10.1 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide * (320 mg, 1.0 mmol) and trifluoroacetic acid (156 μL, 2.0 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 20 mL). The

organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **29** (237 mg, 71%) as a red solid, mp (MeOH/EtOAc) 187–190 °C; ^{1}H NMR δ 8.23 (d, J = 9.1 Hz, 1 H, H-8), 8.10 (d, J = 1.8 Hz, 1 H, H-5), 7.55 (br s, 1 H, NH), 7.36 (dd, J = 9.1, 1.8 Hz, 1 H, H-7), 3.59–3.64 (m, 2 H, CH_2N), 3.11 (sept, J = 6.9 Hz, 1 H, CH), 2.61 (dd, J = 6.2, 6.0 Hz, 2 H, CH_2N), 2.40–2.47 (m, 4 H, 2 × CH_2), 1.56–1.62 (m, 4 H, 2 × CH_2), 1.40–1.47 (m, 2 H, CH_2), 1.33 (d, J = 6.9 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 158.6, 149.8, 138.4, 128.9, 127.2, 121.6, 113.4, 56.7, 54.3 (2), 38.1, 34.9, 26.0 (2), 24.2, 23.2 (2). Anal. calcd for $C_{17}H_{25}N_5O_2 \cdot ¼H_2O$: C, 60.8; H, 7.7; N, 20.9; found C, 61.2; H, 8.1; N, 21.2%.

Example 13

***N*^{1}-(6-*tert*-Butyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (34).**

6-*tert*-Butyl-1,2,4-benzotriazin-3-amine 1-Oxide (31). A mixture of 4-*tert*-butyl-2-nitroaniline (**30**) [Seko, S. et al, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1437] (4.11 g, 21.2 mmol) and cyanamide (3.56 g, 84.6 mmol) were mixed together at 100 °C, cooled to 50 °C, cHCl (15 mL) added carefully and the mixture heated at 100 °C for 4 h. The mixture was cooled to 50 °C, 7.5 M NaOH solution added until the mixture was strongly basic and the mixture stirred at 100 °C for 3 h. The mixture was cooled, diluted with water (200 mL), filtered, washed with water (3 × 50 mL), washed with ether (3 × 30 mL) and dried. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **31** (2.56 g, 62%) as a yellow powder, mp (MeOH/EtOAc) 209–212 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.04 (d, J = 9.0 Hz, 1 H, H-8), 7.45 (dd, J = 9.0, 2.0 Hz, 1 H, H-7), 7.22 (br s, 2 H, NH_2), 7.39 (d, J = 2.0 Hz, 1 H, H-5), 1.32 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR [$(CD_3)_2SO$] δ 160.4, 158.9, 148.8, 127.9, 123.4, 120.8, 119.4, 35.0, 30.2 (3). Anal. calcd for $C_{11}H_{14}N_4O$: C, 60.5; H, 6.5; N, 25.7; found C, 60.8; H, 6.6; N, 25.8%.

6-*tert*-Butyl-3-chloro-1,2,4-benzotriazine 1-Oxide (32). Sodium nitrite (285 mg, 4.1 mmol) was added in small portions to a stirred solution of amine **31** (0.45 g, 2.1 mmol) in trifluoroacetic acid (20 mL) at 5 °C and the solution stirred at 20 °C for 3 h. The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (50 mL) and DMF (0.5 mL) and stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water, stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid

was suspended in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **32** (382 mg, 78%) as a pale yellow solid, mp (EtOAc/DCM) 91–94 °C; ^{1}H NMR δ 8.33 (d, J = 9.1 Hz, 1 H, H-8), 7.93 (d, J = 2.0 Hz, 1 H, H-5), 7.83 (dd, J = 9.1, 2.0 Hz, 1 H, H-7), 1.46 [s, 9 H, C(CH$_3$)$_3$]; ^{13}C NMR δ 161.2, 156.5, 147.1, 131.3, 129.4, 123.3, 119.2, 35.4, 30.1 (3). Anal. calcd for $C_{11}H_{12}ClN_3O$: C, 55.6; H, 5.1; N, 17.7; Cl, 14.9; found C, 55.3; H, 4.9; N, 17.5; Cl, 15.0%.

***N*^{1}-(6-*tert*-Butyl-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (33).** *N*,*N*-Dimethylethanediamine (0.66 mL, 6.0 mmol) was added to a stirred solution of chloride **32** (476 mg, 2.0 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **33** (536 mg, 93%) as a yellow solid, mp (MeOH/EtOAc) 140–144 °C; ^{1}H NMR δ 8.16 (d, J = 9.0 Hz, 1 H, H-8), 7.53 (d, J = 2.0 Hz, 1 H, H-5), 7.35 (dd, J = 9.0, 2.0 Hz, 1 H, H-7), 6.01 (br s, 1 H, NH), 3.56–3.61 (m, 2 H, CH_2N), 2.62–2.65 (m, 2 H, CH_2N), 2.33 [s, 6 H, N(CH$_3$)$_2$], 1.38 [s, 9 H, C(CH$_3$)$_3$]; ^{13}C NMR δ 159.8, 159.2, 149.0, 129.0, 123.8, 121.8, 119.9, 57.5, 45.0 (2), 38.5, 35.5, 30.7 (3). Anal. calcd for $C_{15}H_{23}N_5O\cdot\frac{1}{4}H_2O$: C, 61.3; H, 8.1; N, 23.8; found C, 61.1; H, 8.1; N, 23.8%.

***N*^{1}-(6-*tert*-Butyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (34).** Hydrogen peroxide (70%, 0.84 mL, ca. 16.9 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.4 mL, 16.9 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **33** (489 mg, 1.7 mmol) and trifluoroacetic acid (260 μL, 3.4 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **34** (357 mg, 69%) as a red solid, mp (MeOH/EtOAc) 165–169 °C; ^{1}H NMR δ 8.23–8.26 (m, 2 H, H-5, H-8), 7.55 (dd, J = 9.2, 2.1 Hz, 1 H, H-7), 7.47 (br s, 1 H, NH), 3.61–3.64 (m, 2 H, CH_2N), 2.60 (t, J = 6.0 Hz, 2 H, CH_2N), 2.28 [s, 6 H, (CH$_3$)$_2$], 1.42 [s, 9 H, C(CH$_3$)$_3$]; ^{13}C NMR δ 160.9, 150.0, 138.3, 128.6, 126.0, 121.2,

112.7, 57.5, 45.2 (2), 38.9, 36.1, 36.1 (3). Anal. calcd for $C_{15}H_{23}N_5O_2 \cdot \frac{1}{4}H_2O$: C, 58.1; H, 7.6; N, 22.6; found C, 58.1; H, 7.6; N, 22.5%.

Example 14

6-*tert*-Butyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (36).

6-*tert*-Butyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (35). 2-(1-Piperidinyl)ethylamine (0.57 mL, 4.0 mmol) was added to a stirred solution of chloride **32** (379 mg, 1.6 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give 1-oxide **35** (500 mg, 95%) as a yellow powder, mp (MeOH/EtOAc) 145–148 °C; ^{1}H NMR δ 8.16 (d, J = 9.1 Hz, 1 H, H-8), 7.53 (d, J = 2.0 Hz, 1 H, H-5), 7.34 (dd, J = 9.1, 2.0 Hz, 1 H, H-7), 5.99 (br s, 1 H, NH), 3.52–3.57 (m, 2 H, CH_2N), 2.56–2.60 (m, 2 H, CH_2N), 2.38–2.45 (m, 4 H, 2 × CH_2N), 1.55–1.61 (m, 4 H, 2 × CH_2), 1.41–1.47 (m, 2 H, CH_2), 1.37 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR δ 159.8, 159.1, 149.1, 128.9, 123.7, 121.8, 119.9, 56.8, 54.3 (2), 37.9, 35.5, 30.7 (3), 26.0 (2), 24.4. Anal. calcd for $C_{18}H_{27}N_5O \cdot \frac{1}{4}H_2O$: C, 64.7; H, 8.3; N, 21.0; found C, 64.5; H, 8.5; N, 21.0%.

6-*tert*-Butyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (36). Hydrogen peroxide (70%, 0.53 mL, ca. 10.6 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.5 mL, 10.6 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **35** (350 mg, 1.1 mmol) and trifluoroacetic acid (164 μL, 2.1 mmol) in $CHCl_3$ (10 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (3 × 30 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **36** (182 mg, 50%) as a red powder, mp (MeOH) 181–184 °C; ^{1}H NMR δ 8.21–8.27 (m, 2 H, H-5, H-8), 7.52–7.58 (m, 2 H, NH, H-7), 3.58–3.64 (m, 2 H, CH_2N), 2.63 (dd, J = 6.1, 5.9 Hz, 2 H, CH_2N), 2.40–2.47 (m, 4 H, 2 × CH_2N), 1.57–1.64 (m, 4 H, 2 × CH_2), 1.42–1.48 (m, 2 H, CH_2), 1.41 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR δ 160.9, 150.0,

138.3, 128.6, 126.0, 121.3, 112.6, 56.7, 54.3 (2), 38.2, 36.2, 30.6 (3), 26.0 (2), 24.4. Anal. calcd for $C_{18}H_{27}N_5O_2$: C, 62.4; H, 8.2; N, 20.2; found C, 62.3; H, 7.8; N, 20.2%.

Example 15

3-Ethyl-6-methoxy-1,2,4-benzotriazine 1,4-Dioxide (41).

3-Chloro-6-fluoro-1,2,4-benzotriazine 1-Oxide (38). Sodium nitrite (2.94 g, 42.6 mmol) was added in small portions to a stirred solution of amine **37** [Hay et. al., *J. Med. Chem.* **2003**, 46, 169] (3.84 g, 21.3 mmol) in trifluoroacetic acid (80 mL) at 5 °C and the solution stirred at 20 °C for 3 h. The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (80 mL) and DMF (0.5 mL) and stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water, stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **38** (1.91 g, 45%) as a pale yellow solid, mp (EtOAc) 166–168 °C; ^{1}H NMR δ 8.45 (dd, *J* = 9.5, 5.3 Hz, 1 H, H-8), 7.61 (dd, *J* = 8.3, 2.6 Hz, 1 H, H-5), 7.47–7.52 (m, 1 H, H-7); ^{13}C NMR δ 167.1 (q, *J* = 264 Hz), 158.4, 149.2, 131.0, 123.4 (d, *J* = 11 Hz), 120.1 (d, *J* = 26 Hz), 112.9 (d, *J* = 23 Hz). Anal. calcd for $C_7H_3ClFN_3O$: C, 42.1; H, 1.5; N, 21.1; Cl, 17.8; found C, 42.4; H, 1.6; N, 21.2; Cl, 17.8%.

3-Ethyl-6-fluoro-1,2,4-benzotriazine 1-Oxide (39). $Pd(PPh_3)_4$ (196 mg, 0.17 mmol) was added to a stirred solution of chloride **38** (329 mg, 1.7 mmol) and tetraethyltin (0.7 mL, 3.3 mmol) in DME (20 mL), the solution degassed, and stirred under N_2 at reflux temperature for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/pet. ether to give an oil which was further purified by chromatography, eluting with 5% EtOAc/DCM, to give 1-oxide **39** (295 mg, 93%) as a white solid, mp (EtOAc/pet. ether) 122–124 °C; ^{1}H NMR δ 8.48 (dd, *J* = 9.5, 5.5 Hz, 1 H, H-8), 7.60 (dd, *J* = 8.7, 2.6 Hz, 1 H, H-5), 7.38 (m, 1 H, H-7), 3.04 (q, *J* = 7.6 Hz, 2 H, CH_2), 1.43 (t, *J* = 7.6 Hz, 3 H, CH_3); ^{13}C NMR δ 168.6 (q, *J* = 175 Hz), 165.1, 149.5 (d, *J* = 15 Hz), 130.5, 123.2 (d, *J* = 11 Hz), 120.0 (d, *J* = 26 Hz), 112.7 (d, *J* = 22 Hz), 30.7, 12.2. Anal. calcd for $C_9H_8FN_3O$: C, 56.0; H, 4.2; N, 21.8; found C, 56.0; H, 4.2; N, 21.8%.

3-Ethyl-6-methoxy-1,2,4-benzotriazine 1-Oxide (40). Sodium (55 mg, 2.4 mmol) was added to a stirred solution of fluoride **39** (310 mg, 1.6 mmol) in MeOH (10 mL) and the solution was stirred at 20 °C for 4 h under N_2. The solvent was evaporated and the residue partitioned between DCM (20 mL) and water (20 mL). The organic fraction was dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/pet. ether, to give 1-oxide **40** (156 mg, 56%) as a white solid, mp (EtOAc/ pet. ether) 109–111 °C; ^{1}H NMR δ 8.32 (d, J = 9.5 Hz, 1 H, H-8), 7.24 (dd, J = 9.5, 2.6 Hz, 1 H, H-7), 7.19 (d, J = 2.6 Hz, 1 H, H-5), 3.98 (s, 3 H, OCH_3), 3.00 (q, J = 7.6 Hz, 2 H, CH_2), 1.43 (t, J = 7.6 Hz, 3 H, CH_3); ^{13}C NMR δ 168.8, 165.3, 150.3, 128.5, 122.9, 121.7, 105.8, 56.2, 30.7, 12.2. Anal. calcd for $C_{10}H_{11}N_3O_2$: C, 58.5; H, 5.4; N, 20.5; found C, 58.6; H, 5.4; N, 20.5%.

3-Ethyl-6-methoxy-1,2,4-benzotriazine 1,4-Dioxide (41). Hydrogen peroxide (70%; 0.34 ml, ca. 6.7 mol) was added dropwise to a stirred solution of trifluoroacetic anhydride (0.95 mL, 6.7 mmol) in DCM (15 mL) at 5 °C. The solution was stirred at 20 °C for 5 min then cooled to 5 °C, added to the solution of 1-oxide **40** (138 mg, 0.67 mmol) at 5 °C and the mixture stirred vigorously at 20 °C for 16 h. Dilute aqueous NH_3 solution was added and the mixture stirred at 20 °C for 30 minutes. The mixture was extracted with $CHCl_3$ (5 × 30 mL), the combined organic fraction dried and the solvent evaporated (CAUTION). The residue was purified by chromatography, eluting with 20% EtOAc/DCM, to give 1,4-dioxide **41** (79 mg, 53%) as pale yellow powder, mp (MeOH) 169–171 °C; ^{1}H NMR δ 8.35 (d, J = 9.5 Hz, 1 H, H-8), 7.76 (d, J = 2.7 Hz, 1 H, H-5), 7.36 (dd, J = 9.5, 2.7 Hz, 1 H, H-7), 4.04 (s, 3 H, OCH_3), 3.20 (q, J = 7.4 Hz, 2 H, CH_2), 1.42 (t, J = 7.4 Hz, 3 H, CH_3); ^{13}C NMR δ 165.7, 157.1, 141.5, 129.7, 124.3, 123.4, 97.4, 56.9, 24.1, 9.2. Anal. calcd for $C_{10}H_{11}N_3O_3$: C, 54.3; H, 5.0; N, 19.0; found C, 54.5; H, 5.0; N, 19.1%.

Example 16

***N*^{1}-(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (45).**

3-Chloro-6-methoxy-1,2,4-benzotriazine 1-Oxide (43). Sodium nitrite (7.14 g, 103.4 mmol) was added in portions to a stirred solution of 6-methoxy-1,2,4-benzotriazin-3-amine 1-oxide **52** [Hay et. al., *J. Med. Chem.* **2003**, 46, 169] (9.94 g, 51.7 mmol) in trifluoroacetic acid (50 mL) at 5 °C and the solution stirred at 20 °C for 1 h. The solution was poured into ice/water, filtered, washed with water (2 × 50 mL) and dried. The solid was suspended in $POCl_3$ (80 mL), DMF (2 drops) added, and

the mixture stirred at 100 °C for 3 h. The solution was poured into ice/water, stirred for 20 minutes and filtered. The solid was dissolved in DCM (150 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **43** (7.42 g, 68%) as a pale yellow solid, mp (EtOAc/DCM) 196–199 °C; ^{1}H NMR δ 8.30 (d, J = 9.6 Hz, 1 H, H-8), 7.32 (dd, J = 9.6, 2.7 Hz, 1 H, H-7), 7.19 (d, J = 2.7 Hz, 1 H, H-5), 4.01 (s, 3 H, OCH_3); ^{13}C NMR δ 166.3, 157.8, 150.2, 128.9, 123.9, 121.9, 105.7, 56.5. Anal. calcd for $C_8H_6ClN_3O_2$: C, 45.4; H, 2.9; N, 19.9; Cl, 16.8; found C, 45.2; H, 2.6; N, 19.9; Cl, 16.9%.

***N*^{1}-(6-Methoxy-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (44).** *N*,*N*-Dimethyl-1,2-ethanediamine (1.33 mL, 12.1 mmol) was added to a stirred solution of chloride **43** (0.85 g, 4.04 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 16 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give amine **44** (0.72 g, 68%) which was dissolved in HCl-saturated MeOH, the solvent evaporated and the residue crystallized as a tan solid, mp (MeOH/EtOAc) 236–239 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.68 (br s, 1 H, NH^+Cl^-), 8.07 (d, J = 9.3 Hz, 1 H, H-8), 8.03 (br s, 1 H, NH), 6.95–6.99 (m, 2 H, H-5, H-7), 3.92 (s, 3 H, OCH_3), 3.70–3.76 (m, 2 H, CH_2N), 3.30–3.35 (m, 2 H, CH_2N), 2.81 [d, J = 4.9 Hz, 6 H, $N(CH_3)_2$]; ^{13}C NMR [$(CD_3)_2SO$] δ 164.9, 159.0, 150.4, 125.4, 121.6, 117.3, 104.3, 55.2, 55.0, 42.3 (2), 35.8. Anal. calcd for $C_{12}H_{18}ClN_5O_2$: C, 48.1; H, 6.1; N, 23.4; Cl, 11.8; found C, 48.3; H, 6.1; N, 23.6; Cl, 11.9%.

***N*^{1}-(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (45).** Hydrogen peroxide (70%; 1.1 mL, ca. 22.6 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (3.2 mL, 22.6 mmol) in DCM (15 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **44** (597 mg, 2.3 mmol) and trifluoroacetic acid (350 μL, 4.5 mmol) in $CHCl_3$ (15 mL) at 5 °C. The solution was stirred at 5 °C for 4 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **45** (424 mg, 67%) as a red solid which was dissolved in HCl saturated MeOH, the solvent evaporated and the residue

crystallized to give the hydrochloride, mp (MeOH/EtOAc) 170–174 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.57 (br s, 1 H, NH^+Cl^-), 8.45 (br s, 1 H, NH), 8.17 (d, *J* = 9.6 Hz, 1 H, H-8), 7.39 (d, *J* = 2.6 Hz, 1 H, H-5), 7.22 (dd, *J* = 9.6, 2.6 Hz, 1 H, H-7), 4.01 (s, 3 H, OCH_3), 3.78–3.82 (m, 2 H, CH_2N), 3.33–3.37 (m, 2 H, CH_2N), 2.82 [d, *J* = 4.5 Hz, 6 H, $N(CH_3)_2$]; ^{13}C NMR [$(CD_3)_2SO$] δ 165.6, 150.1, 139.7, 125.7, 123.4, 119.4, 92.5, 56.8, 54.9, 42.3 (2), 36.0. Anal. calcd for $C_{12}H_{18}ClN_5O_3 \cdot 1½H_2O$; C, 42.1; H, 6.2; N, 20.4; found C, 42.0; H, 5.9; N, 20.0%.

Example 17

6-Methoxy-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (47).

6-Methoxy-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1-Oxide (46). 2-(1-Piperidinyl)ethylamine (0.9 mL, 6.0 mmol) was added to a stirred solution of chloride **43** (509 mg, 2.4 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **46** (736 mg, 100%) as a yellow powder, mp (MeOH) 133–135 °C; ^{1}H NMR δ 8.15 (d, *J* = 9.8 Hz, 1 H, H-8), 6.85–6.88 (m, 2 H, H-5, H-7), 6.00 (br s, 1 H, NH), 3.92 (s, 3 H, OCH_3), 3.52–3.56 (m, 2 H, CH_2N), 2.56–2.60 (m, 2 H, CH_2N), 2.38–2.44 (m, 4 H, 2 × CH_2N), 1.56–1.62 (m, 4 H, 2 × CH_2), 1.42–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 165.4, 159.5, 151.5, 126.0, 122.0, 117.6, 104.5, 56.8, 56.0, 54.3 (2), 37.9, 26.0 (2), 24.4. Anal. calcd for $C_{15}H_{21}N_5O_2 \cdot H_2O$: C, 56.1; H, 7.2; N, 21.8; found C, 55.9; H, 7.0; N, 21.7%.

6-Methoxy-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (47). Hydrogen peroxide (70%; 1.0 mL, ca. 19.7 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.8 mL, 19.6 mmol) in DCM (20 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **46** (599 mg, 2.0 mmol) and trifluoroacetic acid (300 μL, 4.0 mmol) in $CHCl_3$ (20 mL) at 5 °C. The solution was stirred at 5 °C for 16 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **47** (369 mg, 58%) as a red solid, mp (MeOH/EtOAc) 171–173 °C; ^{1}H NMR δ 8.21 (d, *J* = 9.6 Hz, 1 H, H-8), 7.59 (br s, 1 H, NH), 7.52 (d, *J* = 2.6 Hz, 1 H, H-5), 7.04 (dd, *J* = 9.6, 2.6 Hz, 1 H, H-7),

4.02 (s, 3 H, OCH_3), 3.60–3.66 (m, 2 H, CH_2N), 2.61–2.65 (m, 2 H, CH_2N), 2.43–2.48 (m, 4 H, 2 × CH_2N), 1.58–1.65 (m, 4 H, 2 × CH_2), 1.42–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 166.1, 150.2, 140.3, 125.6, 123.5, 120.2, 95.0, 55.7, 56.7, 54.3 (2), 38.1, 25.9 (2), 24.3. Anal. calcd for $C_{15}H_{21}N_5O_3 \cdot ¼H_2O$: C, 55.6; H, 6.7; N, 21.6; found C, 55.7; H, 6.3; N, 21.6%.

Example 18

7-Methyl-*N*-[3-(4-Morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (51).

3-Chloro-7-methyl-1,2,4-benzotriazine 1-Oxide (49). A solution of $NaNO_2$ (3.9 g, 56.3 mmol) in water (15 mL) was added dropwise to a stirred suspension of amine **48** [Hay et. al., *J. Med. Chem.* **2003**, 46, 169] (4.95 g, 28.1 mmol) in 2 M HCl (200 mL) at 5 °C and the mixture stirred vigorously for 2 h at 20 °C. The suspension was filtered, the solid dissolved in dilute aqueous NH_3 (150 mL), filtered and the filtrate acidified with cHCl. The suspension was cooled, filtered and the solid washed with water (2 × 10 mL) and dried. The solid (3.76 g, 21.2 mmol) was suspended in dimethylaniline (6.7 mL, 53 mmol) and $POCl_3$ (14 mL, 149 mmol). The mixture was stirred at reflux temperature for 1 h, the resulting solution poured on to ice (300 mL). The suspension was filtered, washed with water (2 × 20 mL), dissolved in EtOAc (200 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **49** (2.99 g, 72%) as a yellow solid, mp (EtOAc/DCM) 176.5–177 °C [lit (Foye et. al., *J. Het. Chem.* **1982**, *19*, 497) mp (toluene) 177–179 °C]; ^1H NMR δ 8.21 (d, J = 2.0 Hz, 1 H, H-8), 7.89 (d, J = 8.6 Hz, 1 H, H-5), 7.81 (dd, J = 8.6, 2.0 Hz, 1 H, H-6), 2.61 (s, 3 H, CH_3).

7-Methyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1-Oxide (50). 3-(4-Morpholinyl)propylamine (1.4 mL, 9.4 mmol) was added to a stirred solution of chloride **49** (738 mg, 3.8 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 8 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0-10%) of MeOH/DCM, to give 1-oxide **50** (1.12 g, 98%) as a yellow powder, mp 158–160 °C; ^1H NMR [$(CD_3)_2SO$] δ 7.94 (d, J = 1.7 Hz, 1 H, H-8), 7.80 (br s, 1 H, NH), 7.63 (dd, J = 8.6, 1.7 Hz, 1 H, H-6), 7.47 (d, J = 8.6 Hz, 1 H, H-5), 3.53–3.57 (m, 4 H, 2 × CH_2O), 3.36–3.39 (m, 2 H, CH_2N), 2.41 (s, 3 H, CH_3), 2.31–2.38 (m, 6 H, 3 × CH_2N), 1.71–1.77 (m, 2 H, CH_2); ^{13}C NMR

[$(CD_3)_2SO$] δ 158.6, 146.8, 137.6, 134.5, 129.6, 125.7, 118.4, 66.1 (2), 55.8, 53.2 (2), 38.9, 25.3, 20.6. Anal. calcd for $C_{15}H_{21}H_5O_2$: C, 59.4; H, 7.0; N, 23.1; found C, 59.7: H, 7.2; N, 23.2%

7-Methyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (51). Hydrogen peroxide (70%, 0.9 mL, ca. 18 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.6 mL, 17.8 mmol) in DCM (7 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stired solution of 1-oxide **50** (540 mg, 1.78 mmol) and TFA (206 μL, 2.7 mmol) in $CHCl_3$ at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with aqueous $KHCO_3$ solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, followed by 1%Et_3N/10%MeOH/DCM, to give 1,4-dioxide **51** (217 mg, 38%) as a red solid, ^{1}H NMR [$(CD_3)_2SO$] δ 8.52 (t, J = 5.6 Hz, 1 H, NH), 8.14 (d, J = 8.8 Hz, 1 H, H 5), 8.00 (d, J = 1.5 Hz, 1 H, H 8), 7.77 (dd, J = 8.8, 1.5 Hz, 1 H, H 6), 3.58–3.62 (m, 4 H, 2 × CH_2O), 3.43–3.47 (m, 2 H, CH_2N), 2.50 (s, 3 H, CH_3), 2.35–2.42 (m, 6 H, 3 × CH_2N), 1.75–1.81 (m, 2 H, CH_2); ^{13}C NMR [$(CD_3)_2SO$] δ 149.4, 137.4, 137.2, 136.7, 129.5, 119.4, 116.6, 66.0 (2), 56.3, 53.2 (2), 40.0, 24.7, 20.7. Anal. calcd for $C_{15}H_{21}H_5O_3 \cdot 2HCl \cdot ½H_2O$: C, 44.9; H, 6.0; N, 17.5; Cl, 17.7; found C, 44.8: H, 6.3; N, 17.0; Cl 16.5%.

Example 19

3-Ethyl-7-(2-methoxyethoxy)-1,2,4-benzotriazine 1,4-Dioxide (58).

3-Amino-1,2,4-benzotriazin-7-ol 1-Oxide (53). A mixture of 4-amino-3-nitrophenol (**52**) (1.0 g, 6.4 mmol) and cyanamide (1.1 g, 26 mmol) were mixed together at 100 °C, cooled to 50 °C, cHCl (10 mL) added carefully and the mixture heated at 100 °C for 4 h. The mixture was cooled to 50 °C, 7.5 M NaOH solution added until the mixture was strongly basic and the mixture stirred at 100 °C for 3 h. The mixture was cooled, diluted with water (200 mL), filtered, washed with water (3 × 50 mL), washed with ether (3 × 30 mL) and dried to give 1-oxide **53** (1.1 g, 97%) as a yellow powder, mp > 300 °C (lit. [Friebe et. al., *US Patent* 5,856,325, Jan 5, 1999] mp (HOAc) >270 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 10.37 (br s, 1 H, OH), 7.48 (dd, J = 7.7, 2.6 Hz, 1 H, H-6), 7.40–7.37 (m, 2 H, H-5, H-8), 6.96 (br s, 2 H, NH_2).

7-(2-Methoxyethoxy)-1,2,4-benzotriazin-3-amine 1-Oxide (54). A mixture of 7-hydroxy-1-oxide **53** (1.00 g, 5.8 mmol), dry K_2CO_3 (2.40 g, 17.4 mmol) and 2-bromoethylmethylether (2.42 g, 17.4 mmol) in DMF (20 mL) was heated at 80 °C for 2 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0–3%) MeOH/DCM, to give compound **54** (1.06 g, 77%) as a yellow powder, mp (DCM/pet. ether) 201 –203°C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.07 (d, J = 9.5 Hz, 1 H, H-5), 7.82 (br s, 2 H, NH_2), 7.76 (dd, J = 9.5, 2.6 Hz, 1 H, H-6), 7.50 (d, J = 2.6 Hz, 1 H, H-8), 4.26, (t, J = 4.3 Hz, 2 H, CH_2), 3.72 (t, J = 4.3 Hz, 2 H, CH_2), 3.33 (s, 3 H, OCH_3). Anal. calcd for $C_{10}H_{12}N_4O_5$: C, 50.8; H, 5.1; N, 23.7; found C, 51.1; H, 5.0; N, 23.7%.

3-Hydroxy-7-(2-methoxyethoxy)-1,2,4-benzotriazine 1-Oxide (55). A suspension of amine **54** (1.00 g, 4.2 mmol) in 2 N HCl (32 mL) was cooled to 5 °C and a solution of $NaNO_2$ (0.58 g, 8.5 mmol) in water (1.5 mL) was added over 1 h. More $NaNO_2$ (0.58 g, 8.5 mmol) in water (1.5 mL) was added and the suspension stirred 72 h at room temperature. The precipitate was filtered and washed with water. The solid was dissolved in 5% aqueous NH_3 and filtered. The filtrate was acidified with conc. HCl to give a precipitate which was filtered, dried and purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give compound **55** (0.68 g, 68%) as a yellow solid, mp (DCM/pet.ether) 190–192 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.52 (br, 1 H, OH), 7.69 (br s, 1 H, H-8), 7.53 (dd, J = 8.8, 2.8 Hz, 1 H, H-6), 7.33 (d, J = 8.8 Hz, 1 H, H-5), 4.19 (t, J = 4.4 Hz, 2 H, CH_2), 3.68 (t, J = 4.4 Hz, 2 H, CH_2), 3.33 (s, 3 H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 154.6, 152.9, 131.8, 129.5, 127.4, 117.8, 101.8, 70.0, 67.9, 58.1. Anal. calcd for $C_{10}H_{11}N_3O_4$: C, 50.6; H, 4.2; N, 17.7; found C, 50.5; H, 4.7; N, 17.7%.

3-Chloro-7-(2-methoxyethoxy)-1,2,4-benzotriazine 1-Oxide (56). A mixture of **55** (1.00 g, 4.3 mmol) in $POCl_3$ (8 mL) was refluxed for 2 h. Excess reagent was evaporated under vacuum, and ice cold water (50 mL) was added to the residue, then solid Na_2CO_3 (1.0 g) was added portionwise. The resulting precipitate was filtered and purified by chromatography, eluting with a gradient (50–100%) of DCM/pet. ether, to give chloride **56** (0.90 g, 83%) as a pale yellow solid, mp (DCM/pet. ether) 121–125 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.00 (d, J = 9.2 Hz, 1 H, H-5), 7.81 (dd, J = 9.2, 2.9 Hz, 1 H, H-6), 7.68 (d, J = 2.8 Hz, 1 H, H-8), 4.35 (t, J = 4.4 Hz, 2 H, CH_2), 3.74 (t, J = 4.4 Hz, 2 H, CH_2), 3.33 (s, 3 H, OCH_3). Anal. calcd for

$C_{10}H_{10}ClN_3O_3$: C, 47.0; H, 3.9; N,16.4, Cl, 13.9; found C, 46.9; H, 4.3; N, 16.4; Cl, 13.7%.

3-Ethyl-7-(2-methoxyethoxy)-1,2,4-benzotriazine 1-Oxide (57). $Pd(PPh_3)_4$ (92 mg, 0.08 mmol) was added to a N_2 purged solution of chloride **56** (260 mg, 1.0 mmol) and tetraethyltin (0.4 mL, 2.0 mmol) in DMF (15 mL). The purged reaction mixture was heated at reflux temperature for 20 h under N_2. The solvent was evaporated and the residue purified by chromatography, eluting with 50% DCM/pet. ether, to give 1-oxide **57** (142 mg, 56%) as a white powder, mp (DCM/pet. ether) 95–97 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.97 (d, J = 9.2 Hz, 1 H, H-5), 7.74 (dd, J = 9.2, 2.9 Hz, 1 H, H-6), 7.68 (d, J = 2.8 Hz, 1 H, H-8), 4.33 (t, J = 4.4 Hz, 2 H, CH_2), 3.74 (t, J = 4.4 Hz, 2 H, CH_2), 3.32 (s, 3 H, OCH_3), 2.75 (q, J = 7.5 Hz, 2 H, CH_2), 1.33 (t, J = 7.6 Hz, 3 H, CH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 164.9, 159.6, 143.1, 133.3, 129.8, 128.7, 98.1, 69.8, 68.2, 58.1, 29.5, 11.9. Anal. calcd for $C_{12}H_{15}N_3O_3$: C, 57.8; H, 6.1; N, 16.9; found C, 57.7; H, 6.1; N, 16.6%.

3-Ethyl-7-(2-methoxyethoxy)-1,2,4-benzotriazine 1,4-Dioxide (58). A pre formed solution of trifluoroperacetic acid [trifluoroacetic anhydride (1.55 mL, 11 mmol) and 70% Hydrogen peroxide (0.53 mL, 11 mmol)] in $CHCl_3$ (10 mL) was added to a solution of 1-oxide **57** (138 mg, 0.55 mmol) in $CHCl_3$ (5 mL). The reaction mixture was stirred at 20 °C for 48 h, partitioned between DCM (10 mL) and H_2O (15 mL), and the aqueous layer was further extracted with DCM (3 × 10 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient of 0–15 % EtOAc/DCM to give 1,4-dioxide **58** (91 mg, 62%) as yellow solid, mp (DCM/hexane) 150–152 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.27 (d, J = 9.5 Hz, 1 H, H-5), 7.98 (dd, J = 9.4, 2.7 Hz, 1 H, H-6), 7.65 (d; J = 2.7 Hz, 1 H, H-8), 4.35 (t, J = 4.4 Hz, 2 H, OCH_2), 3.74 (t, J = 4.4 Hz, 2 H, CH_2O), 3.32 (s, 3 H, OCH_3), 3.01 (q, J = 7.5 Hz, 2 H, CH_2), 1.28 (t, J = 7.5 Hz, 3 H, CH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.5, 153.8, 135.3, 134.9, 127.4, 120.6, 99.8, 69.8, 68.4, 58.1, 22.9, 9.0. Anal. calcd for $C_{12}H_{15}N_3O_4 \cdot ¼H_2O$: C, 54.4; H, 5.8; N, 15.4; found C, 54.4; H, 5.5; N, 15.4%.

Example 20

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(8-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (66).**

3-Chloro-8-methyl-1,2,4-benzotriazine 1-Oxide (60). Sodium nitrite (6.15 g, 89.1 mmol) was added in small portions to a stirred solution of 8-methyl-1,2,4-benzotriazin-3-amine 1-oxide (**59**) [Hay et. al., *J. Med. Chem.* **2003**, *46*, 169] (7.85 g, 44.6 mmol) in trifluoroacetic acid (80 mL) at 5 °C and the solution stirred at 20 °C for 3 h. The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (100 mL) and DMF (0.5 mL) and stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water, stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was dissolved in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **60** (4.25 g, 49%) as a pale yellow solid, mp (EtOAc/DCM) 170–173 °C; ^{1}H NMR δ 7.78–7.82 (m, 2 H, H-5, H-7), 7.47–7.51 (m, 1 H, H-6), 2.98 (s, 3 H, CH_3); ^{13}C NMR δ 156.4, 149.1, 135.7, 134.5, 133.9, 133.1, 126.7, 23.6. Anal. calcd for $C_8H_6ClN_3O$: C, 49.1; H, 3.1; N, 21.5; found C, 49.4; H, 2.9; N, 21.6%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(8-methyl-1-oxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (61).** *N*,*N*-Dimethylethanediamine (530 μL, 4.9 mmol) was added to a stirred solution of chloride **60** (316 mg, 1.6 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **61** (341 mg, 85%) as a yellow solid, mp (MeOH/EtOAc) 121–123 °C; ^{1}H NMR δ 7.48 (dd, *J* = 8.1, 7.1 Hz, 1 H, H-6), 7.41 (d, *J* = 8.1 Hz, 1 H, H-5), 7.00 (d, *J* = 7.1 Hz, 1 H, H-7), 5.86 (br s, 1 H, NH), 3.51–3.57 (m, 2 H, CH_2N), 2.88 (s, 3 H, CH_3), 2.56–2.60 (m, 2 H, CH_2N), 2.29 [s, 6 H, $N(CH_3)_2$]; ^{13}C NMR δ 158.5, 150.7, 134.4, 134.2, 131.1, 127.5, 124.7, 57.6, 45.0 (2), 38.6, 24.0. Anal. calcd for $C_{12}H_{17}N_5O$: C, 58.3; H, 6.9; N, 28.3; found C, 58.0: H, 7.2; N, 28.1%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(8-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (66).** Hydrogen peroxide (70%, 0.85 mL, ca. 17.0 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.4 mL, 17.0 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **61** (421 mg, 1.7 mmol) and trifluoroacetic acid (262 μL, 3.4 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted

with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (3 × 40 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give (i) starting material **61** (78 mg, 18%); and (ii) 1,4-dioxide **66** (212 mg, 47%) as a red solid, mp (MeOH/EtOAc) 151–154 °C; ^{1}H NMR δ 8.20 (d, *J* = 8.7 Hz, 1 H, H-5), 7.67 (dd, *J* = 8.7, 7.2 Hz, 1 H, H-6), 7.40 (br s, 1 H, NH), 7.23 (d, *J* = 7.2 Hz, 1 H, H-7), 3.61–3.64 (m, 2 H, CH_2N), 2.96 (s, 3 H, CH_3), 2.58–2.62 (m, 2 H, CH_2N), 2.30 [s, 6 H, $N(CH_3)_2$]; ^{13}C NMR δ 149.1, 140.0, 135.6, 135.0, 130.7, 129.6, 115.5, 57.5, 45.2 (2), 38.7, 23.7. Anal. calcd for $C_{12}H_{17}N_5O_2.¼MeOH$: C, 54.2; H, 6.7; N, 25.8; found C, 54.2; H, 6.6; N, 25.9%.

Example 21

N^1,N^1-Diethyl-N^2-(8-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (67).

N^1,N^1-Diethyl-N^2-(8-methyl-1-oxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (62). *N*,*N*-Diethylethanediamine (380 μL, 2.7 mmol) was added to a stirred solution of chloride **60** (214 mg, 1.1 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) MeOH/DCM, to give 1-oxide **62** (297 mg, 99%) as a yellow solid, mp (MeOH/EtOAc) 89–91 °C; ^{1}H NMR δ 7.49 (dd, *J* = 8.4, 7.1 Hz, 1 H, H-6), 7.42 (d, *J* = 8.4 Hz, 1 H, H-5), 7.01 (d, *J* = 7.1 Hz, 1 H, H-7), 5.86 (br s, 1 H, NH), 3.49–3.53 (m, 2 H, CH_2N), 2.90 (s, 3 H, CH_3), 2.69 (dd, *J* = 6.1, 5.9 Hz, 2 H, CH_2N), 2.57 (q, *J* = 7.1 Hz, 4 H, 2 × CH_2N), 1.04 (t, *J* = 7.1 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 158.4, 150.7, 134.5, 134.2, 131.1, 127.4, 124.7, 51.3, 46.6 (2), 38.7, 24.0 11.8 (2). Anal. calcd for $C_{14}H_{21}N_5O$: C, 61.1; H, 7.7; N, 25.4; found C, 61.4; H, 7.8; N, 25.3%.

N^1,N^1-Diethyl-N^2-(8-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (67). Hydrogen peroxide (70%, 0.41 mL, ca. 8.2 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.2 mL, 8.2 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **62** (227 mg, 0.8 mmol) and trifluoroacetic acid (320 μL, 4.1 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted

with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (3 × 40 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **67** (148 mg, 62%) as a red gum, ^{1}H NMR δ 8.19 (d, *J* = 8.7 Hz, 1 H, H-5), 7.67 (dd, *J* = 8.7, 7.2 Hz, 1 H, H-6), 7.49 (br s, 1 H, NH), 7.22 (d, *J* = 7.2 Hz, 1 H, H-7), 3.58–3.62 (m, 2 H, CH_2N), 2.96 (s, 3 H, CH_3), 2.76 (dd, *J* = 6.1, 6.0 Hz, 2 H, CH_2N), 2.61 (q, *J* = 7.1 Hz, 4 H, 2 × CH_2N), 1.07 (t, *J* = 7.1 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 149.2, 140.0, 135.6, 135.0, 130.7, 129.5, 115.5, 51.3, 46.8 (2), 38.9, 23.8, 11.8 (2). The hydrochloride salt crystallized as a red powder, mp (MeOH/EtOAc) 148–152 °C. Anal. calcd for $C_{14}H_{22}ClN_5O_2 \cdot ¼H_2O$: C, 51.0; H, 6.9; N, 20.9; found C, 50.8; H, 6.7; N, 20.7%.

Example 22

N^1-(8-Methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dipropyl-1,2-ethanediamine (68).

N^1-(8-Methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dipropyl-1,2-ethanediamine (63). N^1,N^1-Dipropyl-1,2-ethanediamine (770 μL, 5.3 mmol) was added to a stirred solution of chloride **60** (415 mg, 2.1 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) MeOH/DCM, to give 1-oxide **63** (597 mg, 93%) as a yellow solid, mp (MeOH/EtOAc) 91–93 °C; ^{1}H NMR δ 7.48 (dd, *J* = 8.4, 7.1 Hz, 1 H, H-6), 7.41 (d, *J* = 8.4 Hz, 1 H, H-5), 7.00 (d, *J* = 7.1 Hz, 1 H, H-7), 5.80 (br s, 1 H, NH), 3.47–3.52 (m, 2 H, CH_2N), 2.89 (s, 3 H, CH_3), 2.65–2.69 (m, 2 H, CH_2N), 2.39–2.43 (m, 4 H, 2 × CH_2N), 1.42–1.51 (m, 4 H, 2 × CH_2), 0.89 (t, *J* = 7.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 158.5, 150.7, 134.4, 134.2, 131.1, 127.4, 124.7, 55.9 (2), 52.6, 38.8, 24.0, 20.3 (2), 11.9 (2). Anal. calcd for $C_{16}H_{25}N_5O$: C, 63.3; H, 8.3; N, 23.1; found C, 63.4; H, 8.3; N, 22.7%.

N^1-(8-Methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dipropyl-1,2-ethanediamine (68). Hydrogen peroxide (70%, 0.37 mL, ca. 7.0 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.1 mL, 7.0 mmol) in DCM (10 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **63** (252 mg, 0.7 mmol) and trifluoroacetic acid (114 μL, 1.5 mmol) in $CHCl_3$ (10 mL)

at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (3 × 40 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **68** (133 mg, 56%) as a red solid, mp (MeOH/EtOAc) 85–88 °C; ^{1}H NMR δ 8.19 (d, *J* = 8.7 Hz, 1 H, H-5), 7.65 (dd, *J* = 8.7, 7.2 Hz, 1 H, H-6), 7.46 (br s, 1 H, NH), 7.21 (d, *J* = 7.2 Hz, 1 H, H-7), 3.53–3.59 (m, 2 H, CH_2N), 2.96 (s, 3 H, CH_3), 2.71–2.75 (m, 2 H, CH_2N), 2.42–2.47 (m, 4 H, 2 × CH_2N), 1.44–1.53 (m, 4 H, 2 × CH_2), 0.91 (t, *J* = 7.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 149.1, 140.0, 135.6, 134.9, 130.6, 129.5, 115.5, 56.0 (2), 52.5, 39.0, 23.8, 20.5 (2), 11.8 (2). Anal. calcd for $C_{16}H_{25}N_5O_2$: C, 60.2; H, 7.9; N, 21.9; found C, 60.4; H, 7.9; N, 21.0%.

Example 23

8-Methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (69).

8-Methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1-Oxide (64). 2-(1-Piperidinyl)ethylamine (1.26 mL, 8.9 mmol) was added to a stirred solution of chloride **60** (578 mg, 3.0 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **64** (764 mg, 90%) as a yellow powder, mp (MeOH/EtOAc) 137–140 °C; ^{1}H NMR δ 7.48 (dd, *J* = 7.8, 7.8 Hz, 1 H, H-6), 7.41 (br d, *J* = 7.8 Hz, 1 H, H-5), 7.00 (d, *J* = 7.1 Hz, 1 H, H-7), 5.90 (br s, 1 H, NH), 3.52–3.56 (m, 2 H, CH_2N), 2.90 (s, 3 H, CH_3), 2.55–2.59 (m, 2 H, CH_2N), 2.40–2.45 (m, 4 H, 2 × CH_2N), 1.55–1.61 (m, 4 H, 2 × CH_2), 1.41–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 158.4, 150.7, 134.5, 134.2, 131.1, 127.4, 124.7, 57.0, 54.3 (2), 37.9, 26.0 (2), 24.4, 24.0.

8-Methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (69). Hydrogen peroxide (70%; 0.95 mL, ca. 19.1 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.7 mL, 19.1 mmol) in DCM (20 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **64** (548 mg, 1.9 mmol) and trifluoroacetic acid (294 μL, 3.8 mmol) in $CHCl_3$ (20 mL) at 5 °C. The solution was stirred at 5 °C for 16 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the

solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give (i) starting material **64** (141 mg, 26%); and (ii) 1,4-dioxide **69** (253 mg, 43%) as a red solid, mp (MeOH/EtOAc) 126–128 °C; ^{1}H NMR δ 8.20 (d, *J* = 8.7 Hz, 1 H, H-5), 7.67 (dd, *J* = 8.7, 7.2 Hz, 1 H, H-6), 7.57 (br s, 1 H, NH), 7.22 (d, *J* = 7.2 Hz, 1 H, H-7), 3.63–3.67 (m, 2 H, CH_2N), 2.96 (s, 3 H, CH_3), 2.62–2.67 (m, 2 H, CH_2N), 2.44–2.50 (m, 4 H, 2 × CH_2N), 1.57–1.64 (m, 4 H, 2 × CH_2), 1.40–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 149.1, 140.0, 135.6, 135.1, 130.7, 129.6, 115.5, 56.9, 54.3 (2), 37.9, 25.7 (2), 24.2, 23.7. Anal. calcd for $C_{15}H_{21}N_5O_2 \cdot H_2O$: C, 56.1; H, 7.2; N, 21.8; found C, 56.1; H, 6.5; N, 21.3%.

Example 24

***N*-[2-(2,6-Dimethyl-1-piperidinyl)ethyl]-8-methyl-1,2,4-benzotriazin-3-amine 1,4-Dioxide (70).**

***N*-[2-(2,6-Dimethyl-1-piperidinyl)ethyl]-8-methyl-1,2,4-benzotriazin-3-amine 1-Oxide (65).** 2-(2,6-Dimethyl-1-piperidinyl)ethylamine (700 μL, 4.5 mmol) was added to a stirred solution of chloride **60** (352 mg, 1.8 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **65** (489 mg, 82%) as a yellow solid, mp (MeOH/EtOAc) 169–171 °C; ^{1}H NMR δ 7.48 (dd, *J* = 8.4, 7.1 Hz, 1 H, H-6), 7.37 (d, *J* = 8.4 Hz, 1 H, H-5), 7.00 (d, *J* = 7.1 Hz, 1 H, H-7), 5.56 (br s, 1 H, NH), 3.50–3.55 (m, 2 H, CH_2N), 2.86–2.90 (m, 5 H, CH_2N, CH_3), 2.48–2.56 (m, 2 H, CH_2N), 1.63–1.68 (m, 1 H, CH), 1.52–1.58 (m, 2 H, CH_2), 1.32–1.38 (m, 1 H, CH), 1.23–1.30 (m, 2 H, CH_2), 1.20 (d, *J* = 6.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 158.5, 150.7, 134.5, 134.2, 131.1, 127.5, 124.8, 57.2 (2), 47.5, 39.3, 34.3 (2), 24.4, 24.0 (2), 21.6. Anal. calcd for $C_{17}H_{25}N_5O$: C, 64.7; H, 8.0; N, 22.2; found C, 64.7; H, 8.2; N, 22.3%.

***N*-[2-(2,6-Dimethyl-1-piperidinyl)ethyl]-8-methyl-1,2,4-benzotriazin-3-amine 1,4-Dioxide (70).** Hydrogen peroxide (70%, 0.58 mL, ca. 11.5 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.6 mL, 11.5 mmol) in DCM (10 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **65** (382 mg, 1.2 mmol) and trifluoroacetic acid (178 μL, 2.3 mmol) in $CHCl_3$ (10 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted

with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (3 × 40 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **70** (203 mg, 53%) as a red solid, mp (MeOH/EtOAc) 147–150 °C; ^{1}H NMR δ 8.18 (d, J = 8.6 Hz, 1 H, H-5), 7.66 (dd, J = 8.6, 7.3 Hz, 1 H, H-6), 7.21–7.27 (m, 2 H, NH, H-7), 3.55–3.61 (m, 2 H, CH_2N), 2.90–2.96 (m, 5 H, CH_2N, CH_3), 2.49–2.58 (m, 2 H, CH_2N), 1.64–1.69 (m, 1 H, CH), 1.53–1.58 (m, 2 H, CH_2), 1.25–1.40 (m, 3 H, CH, CH_2), 1.19 (d, J = 6.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 149.2, 139.9, 135.7, 135.0, 130.8, 129.6, 115.5, 57.3 (2), 47.3, 39.5, 34.1 (2), 24.4, 23.8 (2), 21.6. Anal. calcd for $C_{17}H_{25}N_5O_2 \cdot ½H_2O$: C, 60.0; H, 7.7; N, 20.6; found C, 59.9; H, 7.6; N, 20.3%.

Example 25

***N*^{1}-(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine Hydrochloride (75).**

3-Chloro-6,7-dimethyl-1-oxido-1,2,4-benzotriazine (72). A mixture of 4,5-dimethyl-2-nitroaniline **71** (5.0 g, 30.1 mmol) and cyanamide (5.06 g, 120 mmol) were mixed together at 100 °C. The mixture was cooled to ca. 50 °C and cHCl (15 mL) added (CAUTION: exotherm) and the resulting solution heated at 100 °C for 1 h. The solution was cooled to ca. 50 °C and 7.5 M NaOH solution (50 mL) added carefully. The suspension was stirred at 100 °C for 2 h, cooled to 20 °C and diluted with water (100 mL). The suspension was filtered, washed with water (2 × 10 mL), washed with ether (2 × 10 mL) and dried. The yellow solid (4.50 g, 23.7 mmol) was suspended in 2 M HCl (250 mL), cooled to 5 °C, and a solution of $NaNO_2$ (3.27 g, 47.3 mmol) in water (20 mL) added dropwise. The mixture was stirred vigorously for 2 h at 20 °C. The suspension was filtered, the solid suspended in dilute aqueous NH_3 (200 mL) and filtered. The filtrate was acidified with cHCl, cooled at 5 °C for 16 h and the precipitate collected. The solid was washed with water (2 × 15 mL) and dried to give the 3-hydroxy-6,7-dimethyl-1,2,4-benzotriazine 1-oxide (1.21 g, 21%) which was used without further characterization. A mixture of the 3-hydroxide (1.21 g, 6.3 mmol), dimethylaniline (2.0 mL, 15.8 mmol) and $POCl_3$ (4.1 mL, 44.3 mmol) was heated at reflux temperature for 1 h. The solution was poured onto ice, stirred and filtered. The solid was dissolved in EtOAc (200 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give the chloride **72** (1.07 g, 81%) as colourless plates, mp 148–149 °C; ^{1}H NMR δ 8.16 (s, 1 H, H-8), 7.72 (s, 1 H, H-5), 2.51 (s, 3 H, CH_3), 2.50 (s, 3 H,

CH_3); ^{13}C NMR δ 156.1, 148.9, 146.3, 142.5, 132.0, 127.4, 119.8, 20.8, 20.5. Anal. calcd for $C_9H_8ClN_3O$: C, 51.6; H, 3.9; N, 20.0; found C, 51.8; H, 3.7; N, 20.2%

***tert*-Butyl 2-[(6,7-Dimethyl-1-oxido-1,2,4-benzotriazin-3-yl)amino]ethylcarbamate (73).** A solution of chloride **72** (842 mg, 4.0 mmol) and *tert*-butyl 2-aminoethylcarbamate (1.4 g, 8.8 mmol) in DME (50 mL) was heated at reflux temperature for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20–50%) of EtOAc/DCM, to give 1-oxide **73** (1.04 g, 78%) as a yellow solid, mp (EtOAc/DCM) 226–228 °C; ^1H NMR [$(CD_3)_2SO$] δ 7.90 (s, 1 H, H-8'), 7.66 (br s, 1 H, NH), 7.39 (s, 1 H, H-5'), 6.88 (t, J = 5.3 Hz, 1 H, NH), 3.33–3.38 (m, 2 H, CH_2N), 3.14–3.18 (m, 2 H, CH_2N), 2.36 (s, 3 H, CH_3), 2.33 (s, 3 H, CH_3), 1.37 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR [$(CD_3)_2SO$] δ 155.8, 155.6, 147.1, 147.8, 134.8, 128.2, 125.2, 118.5, 77.6, 40.9, 39.0, 28.1 (3), 19.9, 19.2. Anal. calcd for $C_{16}H_{23}N_5O_3$: C, 57.6; H, 7.0; N, 21.0; found C, 57.9; H, 7.0; N, 20.8%.

***tert*-Butyl 2-[(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)amino]ethylcarbamate (74).** MCPBA (1.32 g, 5.3 mmol) was added to a stirred suspension of 1-oxide **73** (0.89 g, 2.7 mmol) in DCM (200 mL) and the mixture stirred at 20 °C for 3 h. The resulting solution was diluted with $CHCl_3$ (100 mL) and washed with saturated aqueous $KHCO_3$ solution (100 mL). The aqueous fraction was extracted with $CHCl_3$ (2 × 50 mL), the combined organic fraction dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 40% EtOAc/pet. ether followed by a gradient (0–5%) of MeOH/DCM, to give (i) starting material **73** (423 mg, 47%), spectroscopically identical with the sample prepared above; and (ii) 1,4-dioxide **74** (235 mg, 25%) as a red solid, mp (MeOH/$CHCl_3$) 235–236 °C; ^1H NMR [$(CD_3)_2SO$] δ 8.15 (t, J = 5.8 Hz, 1 H, NH), 7.99 (s, 1 H, H-8'), 7.93 (s, 1 H, H-5'), 6.94 (br s, 1 H, $NHCO_2$), 3.40–3.45 (m, 2 H, CH_2N), 3.17–3.22 (m, 2 H, CH_2N), 2.46 (s, 3 H, CH_3), 2.39 (s, 3 H, CH_3), 1.36 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR [$(CD_3)_2SO$] δ 155.6, 149.5, 147.3, 137.6, 136.7, 128.2, 119.6, 115.7, 77.6, 40.5, 39.0, 28.1 (3), 20.2, 19.2. Anal. calcd for $C_{16}H_{23}N_5O_4$: C, 55.0; H, 6.6; N, 20.0; found C, 55.0; H, 6.7; N, 19.9%.

***N*^{1}-(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine Hydrochloride (75).** A solution of 1,4-dioxide **74** (152 mg, 0.44 mmol) in MeOH (20

mL) was treated with a solution of HCl gas in MeOH (20 mL) and the solution stirred at 50 °C for 2 h. The solvent was evaporated and the residue pumped under vacuum for 2 h. The solid was recrystallized to give 1,4-dioxide **75** (122 mg, 98%) as a red solid, mp (MeOH/EtOAc) 227–228 °C (dec.); ^{1}H NMR [$(CD_3)_2SO$] δ 8.38 (t, J = 6.2 Hz, 1 H, NH), 8.02 (s, 1 H, H-5'), 7.93–7.97 (m, 4 H, H-8', NH_2.HCl), 3.64–3.68 (m, 2 H, CH_2N), 3.05–3.10 (m, 2 H, CH_2N), 2.48 (s, 3 H, CH_3), 2.41 (s, 3 H, CH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 149.6, 147.7, 138.1, 136.7, 128.5, 119.7, 115.7, 39.4, 38.2, 20.2, 19.3. Anal. calcd for $C_{11}H_{16}ClN_5O_2 \cdot ½H_2O$: C, 44.8; H, 5.8; N, 23.8; found C, 45.1; H, 5.7; N, 23.5%.

Example 26

***N*^{1}-(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (82).**

***N*^{1}-(6,7-Dimethyl-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (77).** *N*,*N*-Dimethyl-1,2-ethanediamine (0.3 mL, 2.7 mmol) was added to a stirred solution of chloride **72** (190 mg, 0.9 mmol) in DME (30 mL) and the solution stirred at reflux temperature for 16 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **77** (181 mg, 76%), mp (MeOH) 175–178 °C; ^{1}H NMR δ 8.00 (s, 1 H, H-8), 7.36 (s, 1 H, H-5), 5.82 (br s, 1 H, NH), 3.52–3.56 (m, 2 H, CH_2N), 2.55–2.59 (m, 2 H, CH_2N), 2.38 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3), 2.27 [s, 6 H, $N(CH_3)_2$]; ^{13}C NMR [$(CD_3)_2SO$] δ 158.9, 147.8, 146.9, 135.4, 129.1, 125.7, 119.4, 57.6, 45.1 (2), 38.7, 20.5, 19.8; MS (EI) *m/z* 261 (M^+, 5%), 224 (3), 217 (1), 58 (100); HRMS calcd for $C_{13}H_{19}N_5O$ (M^+) *m/z* 261.1590, found 261.1587.

***N*^{1}-(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (82).** 70% Hydrogen peroxide (0.3 mL, 6.2 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (0.9 mL, 6.2 mmol) in DCM (5 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **77** (162 mg, 0.6 mmol) and trifluoroacetic acid (96 μL, 1.2 mmol) in $CHCl_3$ (10 mL) at 5 °C. The solution was stirred at 5 °C for 4 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a

gradient (0–5%) of MeOH/DCM, to give 1,4-dioxide **82** (106 mg, 62%) as a red solid, mp (MeOH/EtOAc) 169–173 °C; ^{1}H NMR δ 8.08 (s, 1 H, H-8), 8.05 (s, 1 H, H-5), 7.34 (br s, 1 H, NH), 3.59–3.64 (m, 2 H, CH_2N), 2.58–2.62 (m, 2 H, CH_2N), 2.49 (s, 3 H, CH_3), 2.42 (s, 3 H, CH_3), 2.29 [d, J = 4.5 Hz, 6 H, $N(CH_3)_2$]; ^{13}C NMR δ 149.6, 148.1, 138.1, 137.0, 128.8, 120.4, 116.5, 57.5, 45.2 (2), 38.9, 20.8, 19.9. The compound was dissolved in HCl saturated MeOH, the solvent evaporated and the residue crystallized, mp (MeOH/EtOAc) 178–182 °C. Anal. calcd for $C_{13}H_{19}N_5O_2 \cdot 2HCl$: C, 44.6; H, 6.0; N, 20.0; Cl, 20.2; found C, 44.5; H, 6.3; N, 19.8; Cl, 19.5%.

Example 27

N^1-(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-diethyl-1,2-ethanediamine (83).

N^1-(6,7-Dimethyl-1-oxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-diethyl-1,2-ethanediamine (78). *N*,*N*-Diethyl-1,2-ethanediamine (0.54 mL, 3.8 mmol) was added to a stirred solution of chloride **72** (322 mg, 1.5 mmol) in DME (30 mL) and the solution stirred at reflux temperature for 16 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give amine **78** (438 mg, 98%), mp (MeOH/EtOAc) 130–132 °C; ^{1}H NMR δ 8.01 (s, 1 H, H-8), 7.36 (s, 1 H, H-5), 5.87 (br s, 1 H, NH), 3.47–3.52 (m, 2 H, CH_2N), 2.68 (dd, J = 6.1, 5.9 Hz, 2 H, CH_2N), 2.57 (g, J = 7.1 Hz, 4 H, 2 × CH_2N), 2.39 (s, 3 H, CH_3), 2.35 (s, 3 H, CH_3), 1.03 (t, J = 7.1 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 158.9, 147.8, 146.9, 135.3, 129.1, 125.7, 119.4, 51.2, 46.6 (2), 38.7, 20.5, 19.8, 11.8 (2). Anal. calcd for $C_{15}H_{23}N_5O$: C, 62.3; H, 8.0; N, 24.2; found C, 62.4; H, 8.1; N, 24.5%.

N^1-(6,7-Diethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dimethyl-1,2-ethanediamine (83). 70% H_2O_2 (0.57 mL, 11.3 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.6 mL, 11.3 mmol) in DCM (10 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **78** (328 mg, 1.1 mmol) and trifluoroacetic acid (440 μL, 5.7 mmol) in $CHCl_3$ (10 mL) at 5 °C. The solution was stirred at 5 °C for 4 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give 1,4-dioxide **83** (106 mg, 31%) as a red solid, mp

(MeOH/EtOAc) 136–139 °C; ^{1}H NMR δ 8.07 (s, 1 H, H-8), 8.04 (s, 1 H, H-5), 7.46 (br s, 1 H, NH), 3.57–3.61 (m, 2 H, CH_2N), 2.75 (dd, J = 6.2, 6.0 Hz, 2 H, CH_2N), 2.61 (g, J = 7.1 Hz, 4 H, 2 × CH_2N), 2.48 (s, 3 H, CH_3), 2.42 (s, 3 H, CH_3), 1.07 (t, J = 7.1 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 149.6, 148.0, 138.0, 137.0, 128.8, 120.5, 116.5, 51.3, 46.8 (2), 39.0, 20.8, 19.9, 11.9 (2). Anal. calcd for $C_{15}H_{23}N_5O_2 \cdot ¾H_2O$: C, 56.5; H, 7.7; N, 22.0; found C, 56.4; H, 7.4; N, 21.4%.

Example 28

***N*^{1}-(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dipropyl-1,2-ethanediamine (84).**

***N*^{1}-(6,7-Dimethyl-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dipropyl-1,2-ethanediamine (79).** *N*,*N*-Dipropyl-1,2-ethanediamine (0.32 g, 2.2 mmol) was added to a stirred solution of chloride **72** (184 mg, 0.9 mmol) in DME (30 mL) and the solution stirred at reflux temperature for 16 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give amine **79** (273 mg, 98%), mp (MeOH) 92–94 °C; ^{1}H NMR δ 8.02 (s, 1 H, H-8), 7.37 (s, 1 H, H-5), 5.83 (br s, 1 H, NH), 3.48–3.53 (m, 2 H, CH_2N), 2.69 (dd, J = 6.0, 5.8 Hz, 2 H, CH_2N), 2.40–2.44 (m, 4 H, 2 × CH_2N), 2.38 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3), 1.43–1.51 (m, 4 H, 2 × CH_2), 0.89 (t, J = 7.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 158.9, 147.8, 146.9, 135.3, 129.1, 125.7, 119.4, 55.9 (2), 52.6, 38.9, 20.5, 20.3 (2), 19.8, 11.9 (2). Anal. calcd for $C_{17}H_{27}N_5O$: C, 64.3; H, 8.6; N, 20.1; found C, 64.4; H, 8.7; N, 21.7%.

***N*^{1}-(6,7-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dipropyl-1,2-ethanediamine (84).** Hydrogen peroxide (70%; 0.32 mL, ca. 6.3 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (0.9 mL, 6.3 mmol) in DCM (10 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **79** (199 mg, 0.6 mmol) and trifluoroacetic acid (240 μL, 3.1 mmol) in $CHCl_3$ (10 mL) at 5 °C. The solution was stirred at 5 °C for 4 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **84** (65 mg, 31%) as a red solid, mp (MeOH) 139–141 °C; ^{1}H NMR δ 8.05 (s, 1 H, H-5), 8.04 (s, 1 H, H-8), 7.50 (br s, 1 H, NH), 3.57–3.62 (m, 2 H, CH_2N), 2.73–2.78 (m, 2 H, CH_2N), 2.45–2.50 (m, 7 H, 2

× CH_2N, CH_3), 2.41 (s, 3 H, CH_3), 1.46–1.56 (m, 4 H, 2 × CH_2), 0.91 (t, J = 7.3 Hz, 6 H, 2 × CH_3); ^{13}C NMR δ 149.6, 148.0, 138.1, 137.0, 128.8, 120.4, 116.5, 55.9 (2), 52.5, 38.9, 20.8, 20.2 (2), 19.9, 11.8 (2). Anal. calcd for $C_{17}H_{27}N_5O_2$: C, 61.2; H, 8.2; N, 21.0; found C, 61.0; H, 7.9; N, 20.7%.

Example 29

6,7-Dimethyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (85).

6,7-Dimethyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1-Oxide (80). 2-(1-Piperidinyl)ethylamine (0.46 mL, 3.2 mmol) was added to a stirred solution of chloride **72** (272 mg, 1.3 mmol) in DME (30 mL) and the solution stirred at reflux temperature for 16 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give amine **80** (365 mg, 93%), mp (MeOH/EtOAc) 178–180 °C; ^1H NMR δ 8.00 (s, 1 H, H-8), 7.35 (s, 1 H, H-5), 5.97 (br s, 1 H, NH), 3.53–3.58 (m, 2 H, CH_2N), 2.58–2.63 (m, 2 H, CH_2N), 2.43–2.50 (m, 4 H, 2 × CH_2N), 2.38 (s, 3 H, CH_3), 2.35 (s, 3 H, CH_3), 1.57–1.64 (m, 4 H, 2 × CH_2), 1.43–1.49 (m, 2 H, CH_2); ^{13}C NMR δ 158.8, 147.8, 146.9, 135.3, 129.1, 125.7, 119.4, 56.9, 54.3 (2), 37.8, 25.8 (2), 24.3, 20.5, 19.8. Anal. calcd for $C_{16}H_{23}N_5O$: C, 63.8; H, 7.7; N, 23.2; found C, 63.5; H, 8.0; N, 23.2%.

6,7-Dimethyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (85). Hydrogen peroxide (70%; 0.48 mL, ca. 9.6 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.4 mL, 9.6 mmol) in DCM (10 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **80** (290 mg, 1.0 mmol) and trifluoroacetic acid (370 μL, 4.8 mmol) in $CHCl_3$ (10 mL) at 5 °C. The solution was stirred at 5 °C for 4 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give 1,4-dioxide **85** (152 mg, 50%) as a red solid, mp (MeOH/EtOAc) 175–178 °C; ^1H NMR δ 8.07 (s, 1 H, H-8), 8.04 (s, 1 H, H-5), 7.44 (br s, 1 H, NH), 3.60–3.64 (m, 2 H, CH_2N), 2.63 (dd, J = 6.1, 6.0 Hz, 2 H, CH_2N), 2.49 (s, 3 H, CH_3), 2.43–2.47 (m, 4 H, 2 × CH_2N), 2.42 (s, 3 H, CH_3), 1.57–1.64 (m, 4 H, 2 × CH_2), 1.41–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 149.6, 148.1, 138.1, 137.0, 128.8, 120.5,

116.4, 56.9, 54.4 (2), 38.2, 26.0 (2), 24.4, 20.9, 19.9. Anal. calcd for $C_{16}H_{23}N_5O_2 \cdot \frac{1}{4}H_2O$: C, 59.7; H, 7.4; N, 21.7; found C, 59.3; H, 7.3; N, 21.6%.

Example 30

6,7-Dimethyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (86).

6,7-Dimethyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1-Oxide (81). 3-(4-Morpholinyl)propylamine (1.16 mL, 7.9 mmol) was added to a stirred solution of chloride **72** (555 mg, 2.7 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 6 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give amine **81** (788 mg, 94%), mp (MeOH/EtOAc) 145–147 °C; ^{1}H NMR δ 8.00 (s, 1 H, H-8), 7.34 (s, 1 H, H-5), 6.16 (br s, 1 H, NH), 3.72–3.76 (m, 4 H, 2 × CH_2O), 3.55–3.60 (m, 2 H, CH_2), 2.44–2.52 (m, 6 H, 3 × CH_2N), 2.47 (s, 3 H, CH_3), 2.35 (s, 3 H, CH_3), 1.79–1.87 (m, 2 H, CH_2N); ^{13}C NMR δ 159.0, 147.8, 146.9, 135.3, 129.2, 125.7, 119.4, 67.0 (2), 57.3, 53.8 (2), 40.8, 25.2, 20.5, 19.8. Anal. calcd for $C_{16}H_{23}N_5O_2$: C, 60.6; H, 7.3; N, 22.0; found C, 60.6; H, 7.2; N, 22.2%.

6,7-Dimethyl-*N*-[3-(4-morpholinyl)propyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (86). H_2O_2 (70%; 1.0 mL, ca. 20.0 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.8 mL, 20.0 mmol) in DCM (20 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **81** (636 mg, 2.0 mmol) and trifluoroacetic acid (770 μL, 10.0 mmol) in DCM (20 mL) at 5 °C. The solution was stirred at 5 °C for 4 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give (i) starting material **81** (245 mg, 39%) and (ii) 1,4-dioxide **86** (184 mg, 27%) as a red solid, mp (MeOH) 150–152 °C; ^{1}H NMR δ 8.42 (br s, 1 H, NH), 8.06 (s, 1 H, H-5), 8.04 (s, 1 H, H-8), 3.81–3.84 (m, 4 H, 2 × CH_2O), 3.64–3.68 (m, 2 H, CH_2), 2.57 (dd, J = 6.2, 6.0 Hz, 2 H, CH_2N), 2.49–2.54 (m, 4 H, 2 × CH_2N), 2.47 (s, 3 H, CH_3), 2.41 (s, 3 H, CH_3), 1.84-1.90 (m, 2 H, CH_2); ^{13}C NMR δ 149.7, 148.0, 138.0, 137.0, 128.7, 120.5, 116.5, 66.9 (2), 57.8, 53.9 (2), 41.6, 24.4, 20.8,

19.9. Anal. calcd for $C_{16}H_{23}N_5O_3$: C, 57.6; H, 7.0; N, 21.0; found C, 57.3; H, 7.0; N, 20.8%.

Example 31

7-Methoxy-6-methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (91).

7-Methoxy-6-methyl-1,2,4-benzotriazin-3-amine 1-Oxide (88). A mixture 4-methoxy-5-methyl-2-nitroaniline (**87**) [Arnold & McCool. *J. Am. Chem. Soc.* **1942**, *64*, 1315] (2.3 g, 12.6 mmol) and cyanamide (2.0 g, 50 mmol) were mixed together at 100 °C, cooled to 50 °C, cHCl (20 mL) added carefully and the mixture heated at 100 °C for 4 h. The mixture was cooled to 50 °C, 7.5 M NaOH solution added until the mixture was strongly basic and the mixture stirred at 100 °C for 3 h. The mixture was cooled, diluted with water (100 mL), filtered, washed with water (3 × 30 mL), washed with ether (3 × 20 mL) and dried to give crude 1-oxide **88** (2.52 g, 97%) as a yellow powder, mp (MeOH) >320 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.42 (s, 1 H, H-8), 7.39 (d, J = 1.0 Hz, 1 H, H-5), 6.99 (br s, 2 H, NH_2), 3.90 (s, 3 H, OCH_3), 2.30 (s, 3 H, CH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 159.7, 155.4, 144.8, 139.3, 128.4, 126.2, 96.6, 56.0, 16.7.

3-Chloro-7-methoxy-6-methyl-1,2,4-benzotriazine 1-Oxide (89). Sodium nitrite (1.7 g, 24.4 mmol) was added in small portions to a stirred solution of 1-oxide **88** (2.50 g, 12.2 mmol) in trifluoroacetic acid (20 mL) at 5 °C and the solution stirred at 20 °C for 3 h. The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (50 mL) and DMF (0.2 mL) stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water, stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **89** (1.38 g, 50%) as a pale yellow solid, mp (EtOAc/DCM) 200–202 °C; ^{1}H NMR δ 7.70 (d, J = 0.9 Hz, 1 H, H-5), 7.59 (s, 1 H, H-8), 4.03 (s, 3 H, OCH_3), 2.44 (d, J = 0.9 Hz, 3 H, CH_3); ^{13}C NMR δ 160.7, 154.7, 143.5, 142.0, 133.1, 128.4, 96.4, 56.6, 17.4. Anal. calcd for $C_9H_8ClN_3O_2$: C, 47.9; H, 3.6; N, 18.6; found C, 48.1; H, 3.4; N, 18.7%.

7-Methoxy-6-methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1-Oxide (90). 2-(1-Piperidinyl)ethylamine (0.74 mL, 5.2 mmol) was added to a stirred solution of chloride **89** (467 mg, 2.1 mmol) in DME (50 mL) and the solution stirred at

reflux temperature for 4 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give 1-oxide **90** (574 mg, 87%) as a yellow powder, mp (MeOH/EtOAc) 195–197 °C, ^{1}H NMR δ 7.50 (s, 1 H, H-8), 7.36 (d, J = 0.8 Hz, 1 H, H-5), 5.83 (br s, 1 H, NH), 3.92 (s, 3 H, OCH_3), 3.49–3.54 (m, 2 H, CH_2N), 2.54–2.59 (m, 2 H, CH_2N), 2.39–2.43 (m, 4 H, 2 × CH_2N), 2.33 (s, 3 H, CH_3), 1.54–1.60 (m, 4 H, 2 × CH_2), 1.41–1.47 (m, 2 H, CH_2); ^{13}C NMR δ 158.6, 156.3, 145.2, 140.2, 129.4, 126.8, 96.9, 57.0, 56.0, 54.3 (2), 38.0, 26.0 (2), 24.4, 17.2. Anal. calcd for $C_{16}H_{23}N_5O_2$: C, 60.6; H, 7.3; N, 22.1; found C, 60.6; H, 7.1; N, 22.3%.

7-Methoxy-6-methyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (91). Hydrogen peroxide (70%; 0.8 mL, ca. 16.3 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.3 mL, 16.3 mmol) in DCM (20 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **90** (518 mg, 1.6 mmol) and trifluoroacetic acid (250 μL, 3.3 mmol) in $CHCl_3$ (15 mL) at 5 °C. The solution was stirred at 5 °C for 16 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **91** (238 mg, 44%) as red needles, mp (MeOH/EtOAc) 201–205 °C; ^{1}H NMR δ 8.04 (d, J = 1.0 Hz, 1 H, H-5), 7.50 (s, 1 H, H-8), 7.32 (br s, 1 H, NH), 3.97 (s, 3 H, OCH_3), 3.57–3.62 (m, 2 H, CH_2N), 2.59–2.63 (m, 2 H, CH_2N), 2.41–2.48 (m, 7 H, 2 × CH_2N, CH_3), 1.56–1.63 (m, 4 H, 2 × CH_2), 1.41–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 157.9, 149.2, 141.3, 134.2, 129.6, 117.6, 97.7, 56.9, 56.4, 54.4 (2), 38.3, 26.0 (2), 24.4, 17.5. Anal. calcd for $C_{16}H_{23}N_5O_3$: C, 57.6; H, 7.0; N, 21.0; found C, 58.0; H, 7.3; N, 21.4%.

Example 32

N^1-(6-Methoxy-7-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^2,N^2-dimethyl-1,2-ethanediamine (96).

6-Methoxy-7-methyl-1,2,4-benzotriazin-3-amine 1-Oxide (93). A mixture of 5-methoxy-4-methyl-2-nitroaniline (**92**) [James & Felix, U.S. Patent 5,360,986] (8.9 g, 49 mmol) and cyanamide (8.2 g, 196 mmol) were mixed together at 100 °C, cooled to 50 °C, cHCl (50 mL) added carefully and the mixture heated at 100 °C for 4 h. The mixture was cooled to 50 °C, 7.5 M NaOH solution added until the mixture was

strongly basic and the mixture stirred at 100 °C for 3 h. The mixture was cooled, diluted with water (200 mL), filtered, washed with water (3 × 50 mL), washed with ether (3 × 30 mL) and dried. The solid was recrystallised from MeOH to give 1-oxide **93** (6.0 g, 59%) as a yellow powder, mp (MeOH) 289–292 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.91 (d, J = 1.1 Hz, 1 H, H-8), 7.10 (br s, 2 H, NH_2), 6.84 (s, 1 H, H-5), 3.94 (s, 3 H, OCH_3), 2.23 (s, 3 H, CH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 163.7, 160.4, 150.3, 127.3, 124.4, 120.0, 102.7, 56.3, 16.1. Anal. calcd for $C_9H_{10}N_4O_2 \cdot ¼MeOH$: C, 51.9; H, 5.2; N, 26.2; found C, 52.1; H, 4.8; N, 26.4%.

3-Chloro-6-methoxy-7-methyl-1,2,4-benzotriazine 1-Oxide (94). Sodium nitrite (3.38 g, 49.0 mmol) was added in small portions to a stirred solution of 1-oxide **93** (5.05 g, 24.5 mmol) in trifluoroacetic acid (30 mL) at 5 °C and the solution stirred at 20 °C for 3 h. The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (50 mL) and DMF (0.2 mL) stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water, stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **92** (2.72 g, 49%) as a pale yellow solid, mp (EtOAc) 180–182 °C; ^{1}H NMR δ 8.44 (d, J = 0.9 Hz, 1 H, H-8), 7.14 (s, 1 H, H-5), 4.03 (s, 3 H, OCH_3), 2.40 (d, J = 0.9 Hz, 3 H, CH_3); ^{13}C NMR δ 165.4, 156.8, 149.2, 135.5, 128.4, 120.4, 104.3, 56.6, 17.2. Anal. calcd for $C_9H_8ClN_3O_2$: C, 47.9; H, 3.6; N, 18.6; Cl, 15.7; found C, 48.0; H, 3.5; N, 18.6; Cl, 15.7%.

***N*^{1}-(6-Methoxy-7-methyl-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (95).** *N*,*N*-Dimethylethanediamine (0.70 mL, 6.3 mmol) was added to a stirred solution of chloride **94** (474 mg, 2.1 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–15%) MeOH/DCM, to give 1-oxide **95** (529 mg, 90%) as a yellow solid, mp (MeOH) 167–169 °C; ^{1}H NMR δ 7.99 (d, J = 1.0 Hz, 1 H, H-8), 6.79 (s, 1 H, H-5), 5.84 (br s, 1 H, NH), 3.94 (s, 3 H, OCH_3), 3.49–3.54 (m, 2 H, CH_2N), 2.52–2.56 (m, 2 H, CH_2N), 2.27 (d, J = 1.0 Hz, 3 H, CH_3), 2.26 [s, 6 H, $N(CH_3)_2$]; ^{13}C NMR δ 164.5, 159.3, 150.5, 128.5, 125.4, 120.7, 120.0,

57.5, 56.1, 45.1 (2), 38.7, 16.5. Anal. calcd for $C_{13}H_{19}N_5O_2$: C, 56.3; H, 6.9; N, 25.3; found C, 56.5: H, 7.2; N, 25.7%.

***N*^{1}-(6-Methoxy-7-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (96).** Hydrogen peroxide (70%; 0.8 mL, ca. 15.5 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.2 mL, 15.5 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **945** (430 mg, 1.5 mmol) and trifluoroacetic acid (240 μL, 3.1 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 16 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **96** (282 mg, 62%) as a red solid, mp (MeOH/DCM) 169–172 °C; ^{1}H NMR δ 8.05 (d, *J* = 1.0 Hz, 1 H, H-8), 7.46 (s, 1 H, H-5), 7.42 (br s, 1 H, NH), 4.04 (s, 3 H, OCH_3), 3.60–3.64 (m, 2 H, CH_2N), 2.58–2.62 (m, 2 H, CH_2N), 2.32 (d, *J* = 1.0 Hz, 3 H, CH_3), 2.29 [s, 6 H, $(CH_3)_2$]; ^{13}C NMR δ 165.3, 149.8, 139.2, 131.2, 125.2, 122.0, 94.1, 57.5, 56.9, 45.2 (2), 38.8, 16.6. Anal. calcd for $C_{13}H_{19}N_5O_3 \cdot ¼CH_2Cl_2$: C, 50.6; H, 6.3; N, 22.3; found C, 51.0; H, 5.9; N, 22.5%.

Example 33

***N*^{1}-(6,8-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (100).**

3-Chloro-6,8-dimethyl-1,2,4-benzotriazine 1-Oxide (98). A mixture 3,5-dimethyl-2-nitroaniline (**97**) [Andrews et. al., *Aust. J. Chem.* **1972**, *25*, 639] (6.61 g, 39.8 mmol) and cyanamide (6.7 g, 159 mmol) were mixed together at 100 °C, cooled to 50 °C, cHCl (30 mL) added carefully and the mixture heated at 100 °C for 4 h. The mixture was cooled to 50 °C, 7.5 M NaOH solution added until the mixture was strongly basic and the mixture stirred at 100 °C for 3 h. The mixture was cooled, diluted with water (100 mL), filtered, washed with water (3 × 30 mL), washed with ether (3 × 20 mL) and dried to give crude 1-oxide (2.62 g, 35%) as a yellow powder. Sodium nitrite (1.55 g, 22.5 mmol) was added in small portions to a stirred solution of 1-oxide (2.14 g, 11.3 mmol) in trifluoroacetic acid (20 mL) at 5 °C and the solution stirred at 20 °C for 3 h. The solution was poured into ice/water, stirred 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in $POCl_3$ (50 mL) and DMF (0.2 mL) stirred at 100 °C for 1 h. The solution was cooled, poured into ice/water,

stirred for 30 minutes, filtered, washed with water (3 × 30 mL) and dried. The solid was suspended in DCM (150 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **98** (1.58 g, 67%) as a pale yellow solid, mp (EtOAc/DCM) 120–122 °C; ^{1}H NMR δ 7.55 (s, 1 H, H-5), 7.30 (s, 1 H, H-7), 2.93 (s, 3 H, CH_3), 2.52 (s, 3 H, CH_3); ^{13}C NMR δ 156.4, 149.4, 147.7, 135.1, 133.9, 132.2, 125.5, 23.4, 21.9. Anal. calcd for $C_9H_8ClN_3O$: C, 51.6; H, 3.9; N, 20.0; Cl, 16.9; found C, 51.8; H, 3.6; N, 20.2; Cl, 16.6%.

***N*^{1}-(6,8-Dimethyl-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (99).** *N*,*N*-Dimethylethanediamine (0.64 mL, 5.9 mmol) was added to a stirred solution of chloride **98** (494 mg, 2.4 mmol) in DME (80 mL) and the solution stirred at reflux temperature for 2 h. The solution was cooled, the solvent evaporated and the residue partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1-oxide **99** (561 mg, 91%) as a yellow solid, mp (MeOH) 176–179 °C; ^{1}H NMR δ 7.20 (br s, 1 H, H-5), 6.84 (br s, 1 H, H-7), 5.76 (br s, 1 H, NH), 3.50–3.54 (m, 2 H, CH_2N), 2.85 (s, 3 H, CH_3), 2.52–2.56 (m, 2 H, CH_2N), 2.38 (s, 3 H, CH_3), 2.26 [s, 6 H, $N(CH_3)_2$]; ^{13}C NMR δ 158.7, 150.9, 145.6, 133.7, 129.6, 129.4, 123.7, 57.6, 45.1 (2), 38.7, 23.8, 21.6. Anal. calcd for $C_{13}H_{19}N_5O$: C, 59.8; H, 7.3; N, 26.8; found C, 60.0: H, 7.6; N, 27.0%.

***N*^{1}-(6,8-Dimethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (100).** Hydrogen peroxide (70%, 0.80 mL, ca. 15.4 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.2 mL, 15.4 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **99** (403 mg, 1.5 mmol) and trifluoroacetic acid (238 μL, 3.1 mmol) in $CHCl_3$ (20 mL) at 5 °C and the mixture stirred at 20 °C for 4 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of MeOH/DCM, to give 1,4-dioxide **100** (289 mg, 68%) as a red powder, mp (MeOH/EtOAc) 188–192 °C; ^{1}H NMR δ 7.98 (s, 1 H, H-5), 7.35 (br s, 1 H, NH), 7.05 (s, 1 H, H-7), 3.58–3.64 (m, 2 H, CH_2N), 2.92 (s, 3 H, CH_3), 2.57–2.60 (m, 2 H, CH_2N), 2.50 (s, 3 H, CH_3), 2.29 [s, 6 H,

$N(CH_3)_2$]; ^{13}C NMR δ 149.2, 147.0, 139.9, 135.2, 131.7, 129.1, 114.4, 57.6, 45.2 (2), 38.8, 26.6, 22.1. Anal. calcd for $C_{13}H_{19}N_5O_2$: C, 56.3; H, 6.9; N, 25.3; found C, 56.1: H, 7.2; N, 25.1%.

Example 34

6,8-Dimethyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (102).

6,8-Dimethyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1-Oxide (101). 2-(1-Piperidinyl)ethylamine (0.67 mL, 4.7 mmol) was added to a stirred solution of chloride **98** (395 mg, 1.9 mmol) in DME (50 mL) and the solution stirred at reflux temperature for 2 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and dilute aqueous NH_3 (50 mL). The organic fraction was dried and the solvent evaporated. The residue was chromatographed, eluting with a gradient (0-5%) of MeOH/DCM, to give 1-oxide **101** (517 mg, 91%) as a yellow powder, mp (MeOH) 177–178 °C, ^1H NMR δ 7.20 (s, 1 H, H-5), 6.84 (s, 1 H, H-7), 5.84 (br s, 1 H, NH), 3.49–3.55 (m, 2 H, CH_2N), 2.85 (s, 3 H, CH_3), 2.54–2.57 (m, 2 H, CH_2N), 2.39–2.44 (m, 4 H, 2 × CH_2N), 2.37 (s, 3 H, CH_3), 1.55–1.61 (m, 4 H, 2 × CH_2), 1.41–1.47 (m, 2 H, CH_2); ^{13}C NMR δ 158.6, 150.9, 145.6, 133.7, 129.5, 129.4, 123.7, 57.0, 54.3 (2), 37.9, 26.0 (2), 24.4, 23.8, 21.7. Anal. calcd for $C_{16}H_{23}N_5O$: C, 63.8; H, 7.7; N, 23.2; found C, 63.9; H, 8.0; N, 23.5%.

6,8-Dimethyl-*N*-[2-(1-piperidinyl)ethyl]-1,2,4-benzotriazin-3-amine 1,4-Dioxide (102). H_2O_2 (70%; 0.74 mL, ca. 14.9 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.1 mL, 14.9 mmol) in DCM (20 mL) at 5 °C. The solution was stirred at 5 °C for 5 min, warmed to 20 °C for 10 min, then cooled to 5 °C and added to a stirred solution of 1-oxide **101** (448 mg, 1.5 mmol) and trifluoroacetic acid (229 μL, 3.0 mmol) in $CHCl_3$ (15 mL) at 5 °C. The solution was stirred at 5 °C for 16 h, diluted with dilute aqueous NH_3 solution (10 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic fraction was dried and the solvent evaporated. The residue was chromatographed, eluting with a gradient (0-10%) of MeOH/DCM, to give 1,4-dioxide **102** (297 mg, 62%) as a red powder, mp (MeOH/DCM) 195–199 °C; ^1H NMR δ 7.98 (s, 1 H, H-5), 7.43 (br s, 1 H, NH), 7.05 (s, 1 H, H-7), 3.58–3.64 (m, 2 H, CH_2N), 2.92 (s, 3 H, CH_3), 2.59–2.63 (m, 2 H, CH_2N), 2.49 (s, 3 H, CH_3), 2.41–2.46 (m, 4 H, 2 × CH_2N), 1.57–1.64 (m, 4 H, 2 × CH_2), 1.41–1.48 (m, 2 H, CH_2); ^{13}C NMR δ 149.2, 147.0, 139.9, 135.2, 131.6, 129.1, 114.4, 56.9, 51.4 (2), 38.1, 26.0 (2),

24.4, 23.6, 22.1. Anal. calcd for $C_{16}H_{23}N_5O_2$: C, 60.6; H, 7.3; N, 22.1; found C, 60.7; H, 7.6; N, 22.2%.

Table 4 Data for compounds of the invention.

Table 4 lists the characteristics results of compounds of Formula I of this invention, compared with the results of TPZ.

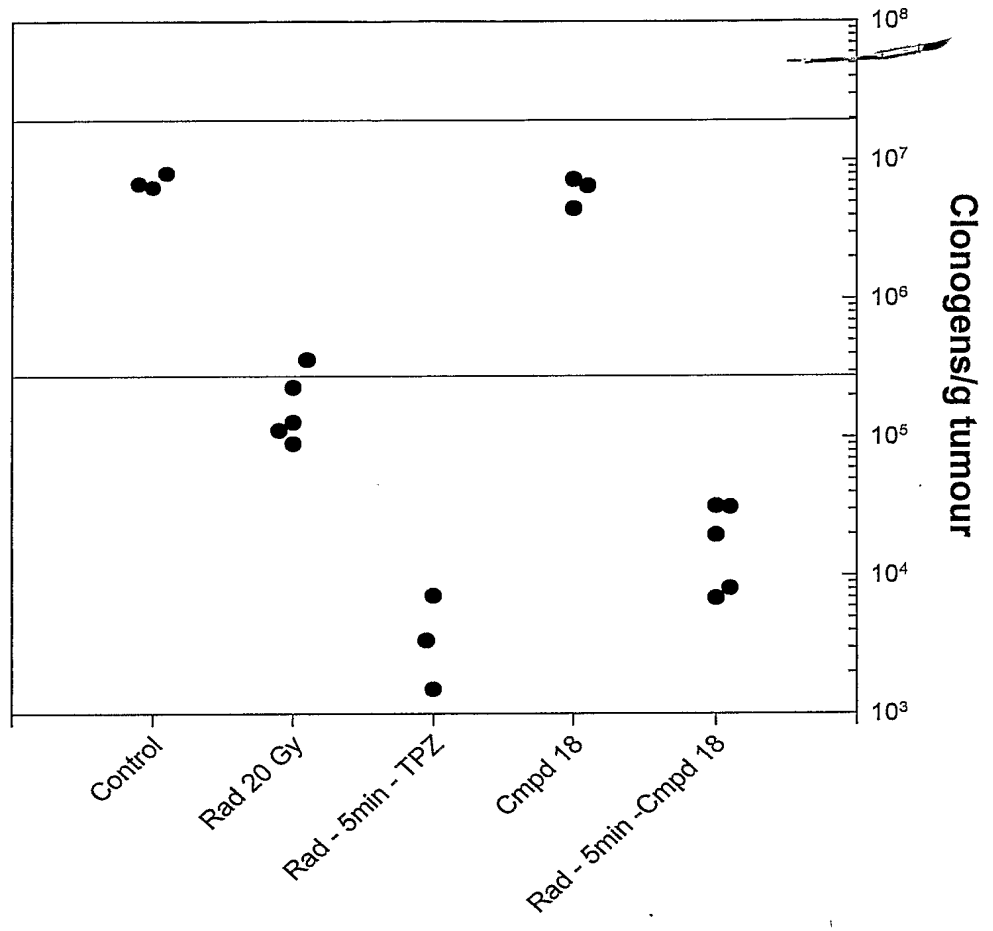
Compound no	A_1	A_2	B	Sol[a] (mM)	HT29 IC50 anoxic[b] (μM)	HT29 HCR[c]	PHD[d] (μm)	AUC_f (μM-hr)	AUC_f/ IC_{50}(hr)
TPZ	H	H	NH_2	8.9	5.8	71.0	43.7	148.1[e]	25.5
3	H	H	$NHCH_2CH_2CN$	2.2	7.0	51.1	52.3	115.5	16.5
5	H	H	$NHCH_2CO_2Et$	6.4	10.0	27.4	54.0	65.4	6.5
9	6-Me	H	CH_2CH_3	4.2	11.4	63.4	112.2	256.7	22.5
12	6-Me	H	CH_2CH_2OH	6.5	4.3	82.4	51.8	87.2	20.3
18	6-Me	H	$NHCH_2CH_2NMe_2$	52.0	4.1	215.0	27.5	69.0[e]	16.8
21	6-Me	H	$NHCH_2CH_2$-PIP	45.1	4.0	57.9	39.0	13.8[e]	3.5
22	6-Me	H	$NHCH_2CH_2$-(PIP)Me_2	48.3	2.8	126.0	46.7	18.4[e]	6.6
34	6-tBu	H	$NHCH_2CH_2NMe_2$	38.4	2.9	142.7	42.5	9.7[e]	3.3
45	6-MeO	H	$NHCH_2CH_2NMe_2$	45.5	14.4	36.2	51.9	239.5	16.6
47	6-MeO	H	$NHCH_2CH_2$-PIP	44.7	10.8	24.4	90.4	152.3	14.1
51	7-Me	H	$NH(CH_2)_3$NMorph	50.0	15.9	23.3	66.8	82.2	5.2
58	7-$O(CH_2)_2OMe$	H	CH_2CH_3	2.1	4.7	68.6	126.9	470.3	100.1
66	8-Me	H	$NHCH_2CH_2NMe_2$	45.6	2.3	163.4	42.2	12.3	5.3
69	8-Me	H	$NHCH_2CH_2$-PIP	55.0	3.8	55.6	53.8	16.1	4.2
74	6-Me	7-Me	$3NHCH_2CH_2NH_2$	24.2	3.0	31.0	49.1	44.8	14.9
82	6-Me	7-Me	$NHCH_2CH_2NMe_2$	47.6	6.6	28.0	46.6	39.4	6.0
85	6-Me	7-Me	$NHCH_2CH_2$-PIP	48.0	2.4	98.9	61.5	13.5	5.6
86	6-Me	7-Me	$NH(CH_2)_3$NMorph	49.0	14.2	20.5	139.1	174.4	12.3
90	6-Me	7-MeO	$NHCH_2CH_2$-PIP	46.6	3.0	67.2	56.3	11.2[e]	3.7
95	6-MeO	7-Me	$NHCH_2CH_2NMe_2$	53.0	33.8	33.1	107.7	280.3	8.3
100	6-Me	8-Me	$NHCH_2CH_2NMe_2$	37.9	14.1	98.9	88.6	91.6	6.5

[a]Solubility in αMEM culture medium. [b]Concentration of drug for 50% inhibition of cell proliferation under hypoxic (0% O_2) conditions. [c]Hypoxic Cytotoxicity Ratio; IC_{50}(20% O_2)/IC_{50}(0% O_2). [d]Penetration Half Distance. [e]Measured AUC_f; other AUC values are by calculation.

Example 35 HT29 Excision assay

Compounds were evaluated with single dose radiation using s.c. HT29 tumors (average of two largest diameters 7-10 mm) grown by inoculating 10^7 cells (obtained by enzymatic dissociation of multicellular spheroids). Drugs were administered as single i.p. doses at their MTD with the following groups in each experiment. A: vehicle control, B: test drug, C: Radiation (20 Gy, cobalt-60, whole body irradiation), D: TPZ (316 μmol/kg) 5 min after radiation, E: Test drug 5 min after radiation. Each group included 3 (A,B) or 5 (C-E) mice. Tumors were excised 18 hr after treatment and plated to determine clonogenicity. Hypoxic cytotoxicity is determined by the difference in surviving fraction between groups C and E, while comparison of A and B evaluates oxic cell killing. Total yield of clonogens was used as the key parameter if cell yields were affected by treatment. Results of this assay are illustrated for compound **18** (and TPZ) in Figure 4. Compound 18 is predicted to be active because it meets all the desired characteristics of a TPZ analogue of this invention (see Table 2), and is demonstrated to have significant activity against hypoxic (radioresistant) cells in HT29 cells ($p < 0.01$relative to radiation only).

Example 36 In vivo activity of compound 18



Activity of compound **18** against hypoxic cells in HT29 tumour xenografts. Animals were treated with radiation alone (RAD, 20 Gy whole body); RAD + TPZ (316 μmol/kg); compound **18** (562 μmol/kg); RAD + compound **18** (562 μmol/kg). Tumours were excised 18 hr after treatment and clonogenic survival determined by staining colonies 14 days later. Each symbol represents a separate tumour. $p<0.01$ (one way ANOVA with Dunnett's test) for RAD + compound **18** and for RAD + TPZ versus RAD only. Horizontal lines are the historical means (solid) and 95% confidence limits (dashed) for untreated controls (upper set) and 20 Gy radiation only (lower set).

Wherein the foregoing description reference has been made to reagents, or integers having known equivalents thereof, then those equivalents are herein incorporated as if individually set forth.

While this invention has been described with reference to certain embodiments and examples, it is to be appreciated that further modifications and variations can be made to embodiments and examples without departing from the spirit of the invention.

AUCKLAND UNISERVICES LIMITED

By Its Attorneys
BALDWIN SHELSTON WATERS

Figure 1

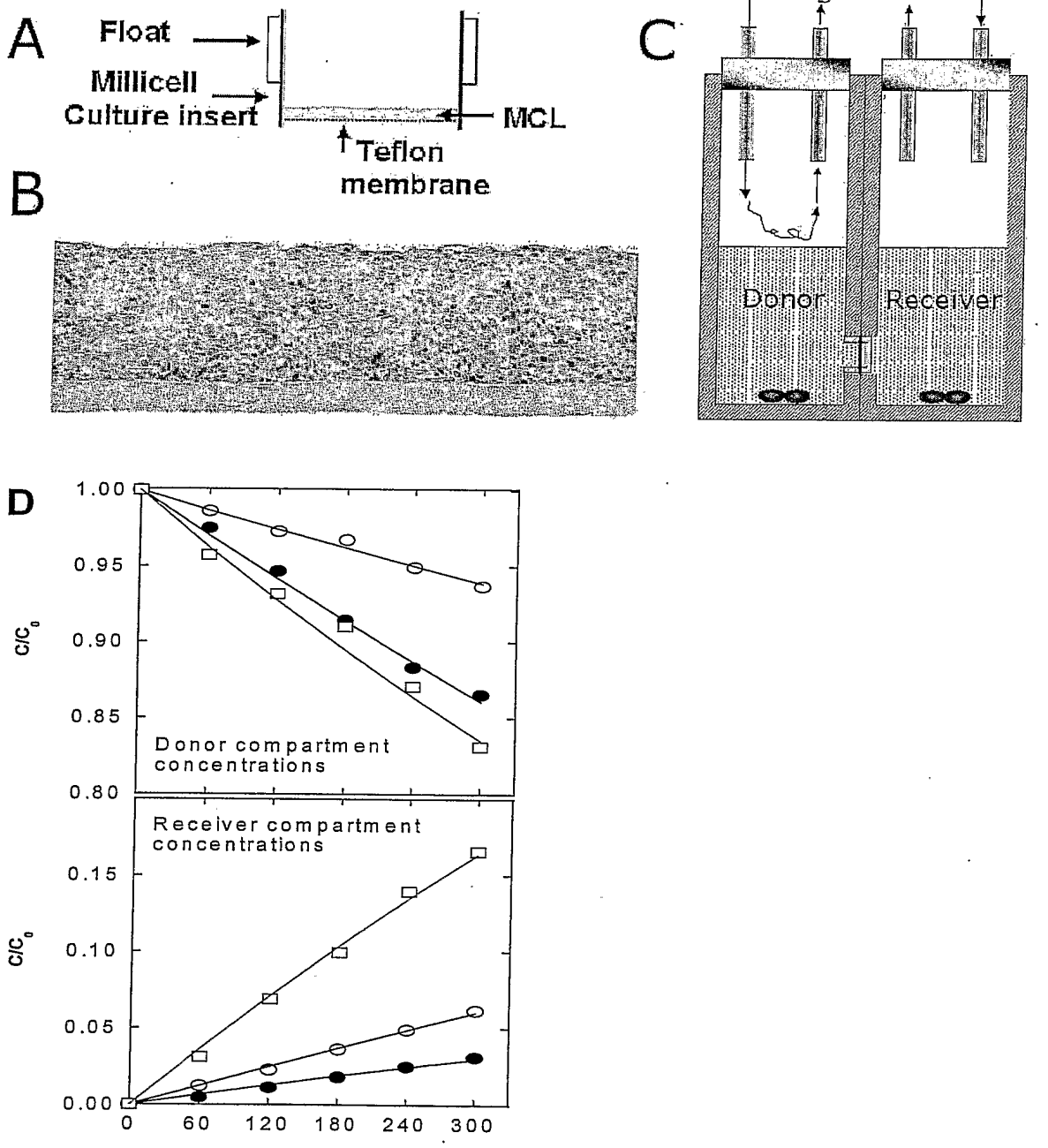

A
Float
Millicell
Culture insert
MCL
Teflon
membrane
B
C
Gas in
Gas out
Gas in
Donor
Receiver
D
1.00
0.95
0.90
0.85
0.80
C/C0
Donor compartment
concentrations
Receiver compartment
concentrations
0.15
0.10
0.05
0.00
C/C0
0
60
120
180
240
300

Figure 2

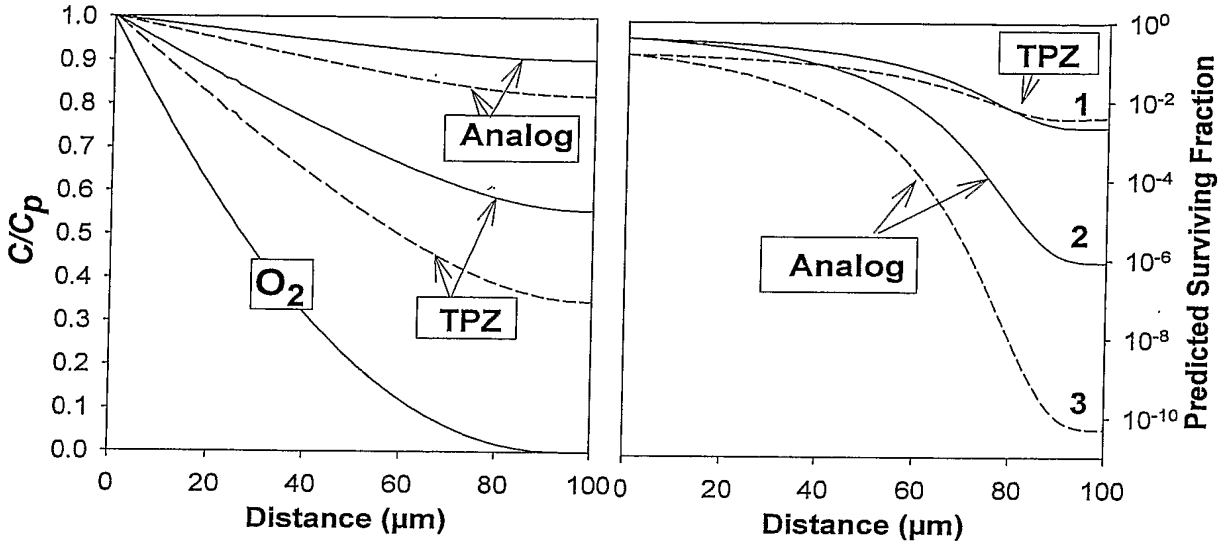
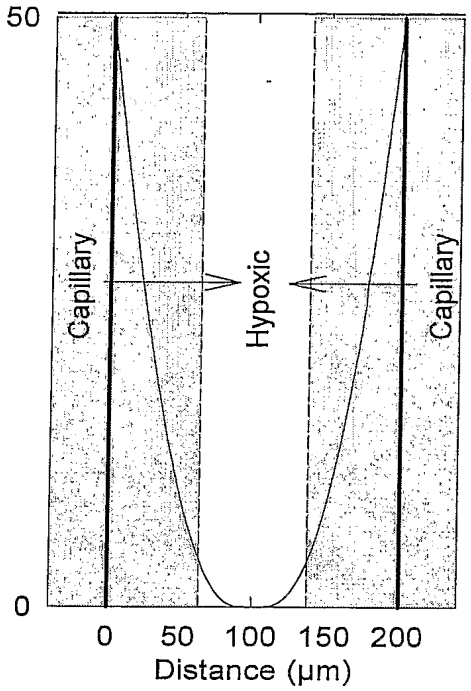
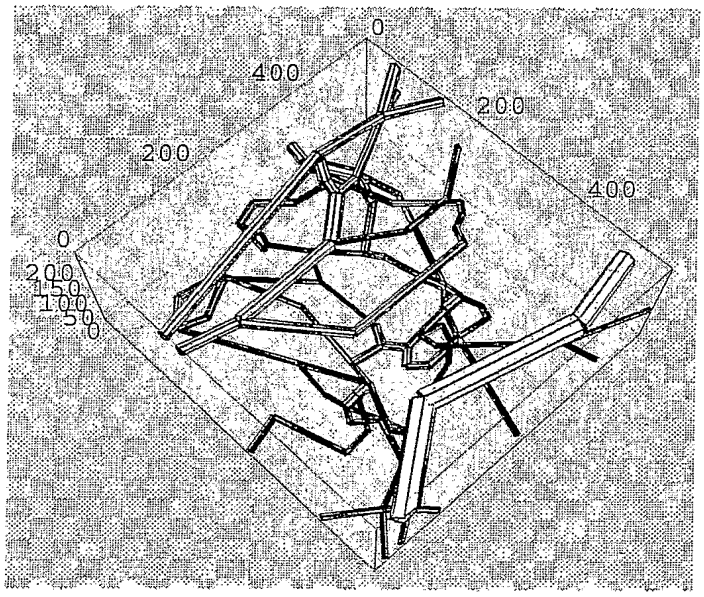

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
C/Cp
Analog
O2
TPZ
0
20
40
60
80
100
Distance (μm)
TPZ
1
2
3
Analog
10^0
10^-2
10^-4
10^-6
10^-8
10^-10
Predicted Surviving Fraction
0
20
40
60
80
100
Distance (μm)

Figure 3.


50
0
Capillary
Hypoxic
Capillary
0
50
100
150
200
Distance (μm)

A


0
400
200
200
400
0
200
150
100
50
0

B

Figure 4.